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14. ABSTRACT
Recently we identified splice variants in whole blood than can distinguish early stage Parkinson's disease (PD) patients from healthy and neurodegenerative controls. The proposed studies will test the hypothesis that the biomarkers will be useful for identifying individuals at risk for PD and for identifying signaling pathways that are disrupted in PD. We are testing the expression of the biomarkers in RNA prepared from whole blood of hyposmic participants in the Parkinson's Associated Risk Study (PARS) (Technical Objective 1.0). In order to establish a model for testing the function of the biomarkers, we are determining whether their expression is altered in human olfactory neurosphere-derived (hONS) cells derived from idiopathic PD patients and healthy controls (Technical Objective 2.0). To determine the signaling pathways affected in PD and identify additional biomarkers, we are using network analysis (Technical Objective 3.0).
During year 1, RNA and cDNA was prepared from PARS samples from Year 0 (baseline) and Year 2. Quantitative polymerase chain reactions were initiated for two of the biomarkers (*APP*, *HNF4*α). Expression of the biomarkers was tested in hONS. We also developed network approaches to reveal the common molecular pathways involved with PD and type 2 diabetes (T2DM). Using these networks, we identified *APP*, *HNF4A* and *SOD2* mRNAs as blood biomarkers predictive of early stage PD. In addition, we determined that *HNF4A* mRNA may be a progression marker in blood for PD.

15. SUBJECT TERMS
Parkinson's disease, biomarkers, neurodegeneration, network and pathway analysis

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INTRODUCTION

Parkinson's disease (PD) is a chronic debilitating disease. Most cases of PD are idiopathic suggesting environmental factors and genetic susceptibility play a role in disease onset. When a patient first experiences motor symptoms, 60% or more of the neurons in the substantia nigra pars compacta have already died and therefore the disease is irreversible. In this regard, early detection of PD, ideally before the onset of motor symptoms, could improve disease management. Recently, we identified and verified 13 mRNA biomarkers in whole blood that can be used to distinguish early stage PD patients from healthy and neurodegenerative controls. Gene network prediction analysis identified a regulatory network connecting the biomarkers with nodes centered on transcription factors that play a role in insulin resistance. In this study we will test the hypothesis that the biomarkers are useful for identifying individuals at risk for PD. Since one of the early signs of PD is hyposmia, we will test the biomarkers in participants of the Parkinson's Associated Risk Study (PARS) who are hyposmic. We will also use human olfactory neurosphere-derived (hONS) cells derived from idiopathic PD patients and healthy controls to examine the expression of the biomarkers in order to identify signaling pathways involved in PD.

BODY

Objective 1. To determine whether the splice variant-specific biomarkers can identify individuals at risk for PD.

- A conference call was held on 29-May-2013 in which the PI discussed the PARS samples with Ken Marek, Clemens Scherzer, Carol Cioffi, Andrew Siderowf and Danna Jennings. It was decided that RNA isolated from whole blood from 100 normosmic individuals (7 with abnormal dopamine transporter (DAT) scans) and 203 hyposmic individuals (57 with abnormal DAT scans) will be sent from Dr. Scherzer's lab to the Dr. Potashkin's lab. The PI will be blinded to the clinical findings. Samples from 2 time points will be included (baseline and year 2).
- RNA was isolated from whole blood from normosmic and hyposmic individuals (269 total) from year 1 baseline samples of the PARS study in Dr. Scherzer's lab. These samples were shipped to Dr. Potashkin's lab in September.
- RNA was isolated from blood from year 2 samples of the PARS study in Dr. Scherzer's lab. 40 of the samples had low RNA integrity values and therefore cannot be used in PCR assays. RNA was re-isolated from the 40 of the samples that initially had low RNA integrity values. The samples were shipped to Dr. Potashkin's lab and received on 19-Feb-2014.
- cDNAs have been prepared on RNA samples from Years 1 and 2 in Dr. Potashkin's lab.
- qPCR assays have begun on the Year 1 samples. Two sets of PCR assays have been run to determine the relative expression levels of *APP* and *HNF4A*. A third set will be run in the second year of funding.
- In order to test the PD biomarkers we previously identified in a separate cohort of study participants we obtained samples from the Harvard NeuroDiscovery Center Biomarker Study. The results showed that expression of seven out of thirteen candidate biomarkers was dysregulated in whole blood of patients with PD compared to healthy controls (Santiago et al, 2013). Published 20-Sep-2013.

Objective 2. To determine whether the expression of the biomarkers is altered in hONS cells prepared from PD patients compared to healthy controls.

- A subcontract was established with Stephen Wood, National Centre for Adult Stem Cell Research, Eskitis Institute, Griffith University, Brisbane, Australia. 19-Jun-2013. The plan for these studies is to use RNA prepared from human olfactory neurosphere-derived cells (hONS) obtained from 8 idiopathic PD patients and 8 healthy controls.
- The RNA from hONS was reverse transcribed in Dr. Wood's lab to produce cDNA and shipped to Dr. Potashkin's lab in September.
- Jose Santiago, the research associated in Dr. Potashkin's lab, quantified the expression of the PD biomarkers in the hONS. The results presented in the figure 1 below indicate that all the markers are expressed in hONS. Only znf160, ptpn1 and map4k1 are differentially express in hONS prepared from PD patients compared to healthy controls. These results suggest that the hONS may be a useful model in which the function of these three markers may be studied.

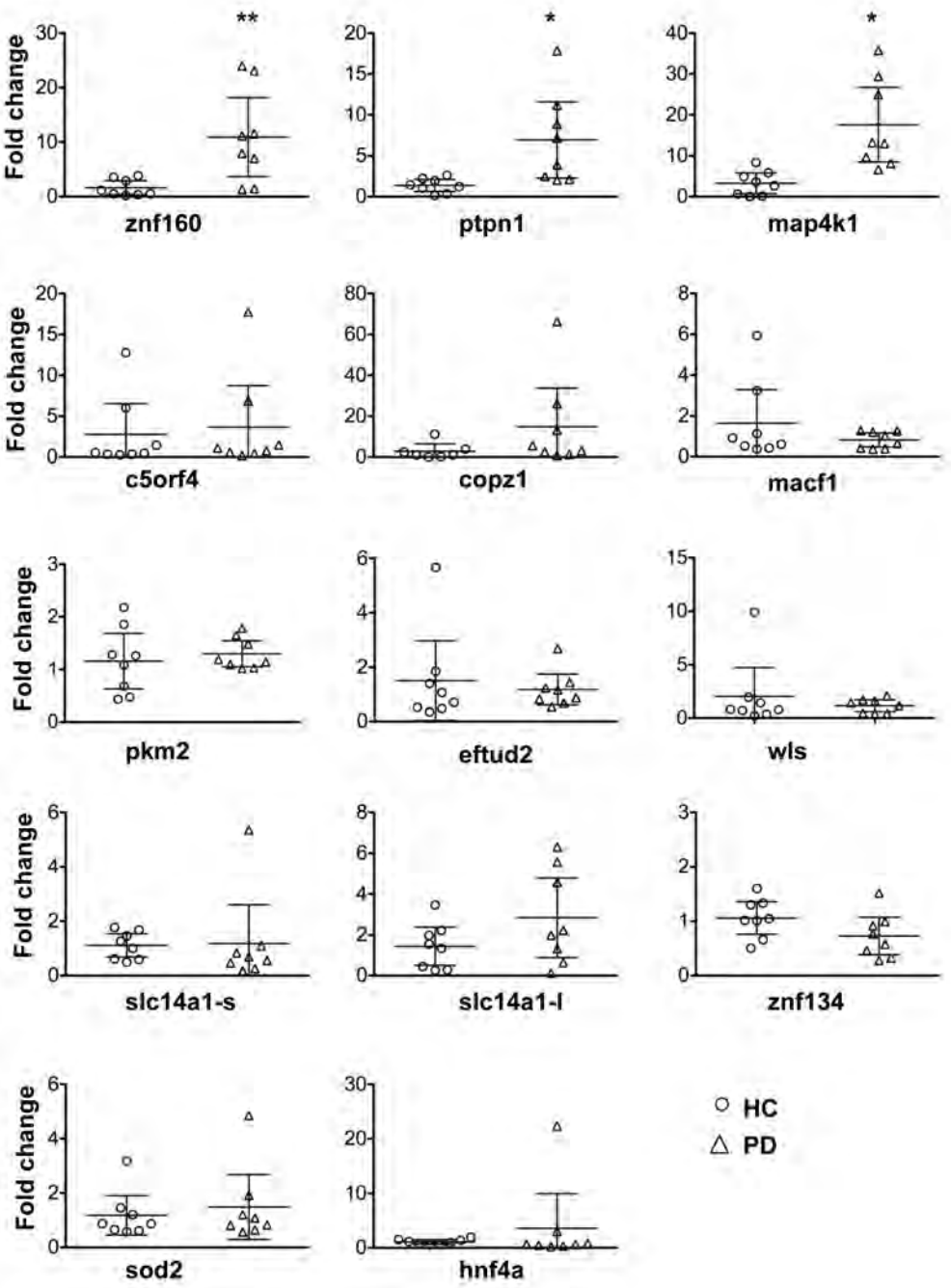

Figure 1. **Relative mRNA expression level of PD biomarkers in HONS cells.** Fold change expression of RNA biomarkers in HONS cells from Parkinson's disease patients (n=8, triangles) compared to healthy controls (n=8, circles). Fold change was calculated using gapdh as a reference gene and expression levels in the healthy controls as a calibrator. Error bars represent the 95% confidence interval. * P<0.01, ** P<0.001

- Dr. Wood performed preliminary silencing RNA (siRNA) experiments studies in hONS on znf160, ptpn1 and map4k1. For these studies antibodies and siRNAs (Dharmacon) were tested on cell line #2801. In order to check that the Dharmacon reagents were working and that hONS cells were susceptible to siRNA knockdown we included siRNAs against Usp9x, since these siRNAs work in human keratinocyte cell line (HaCaT cells). The results are presented in Figure 2.

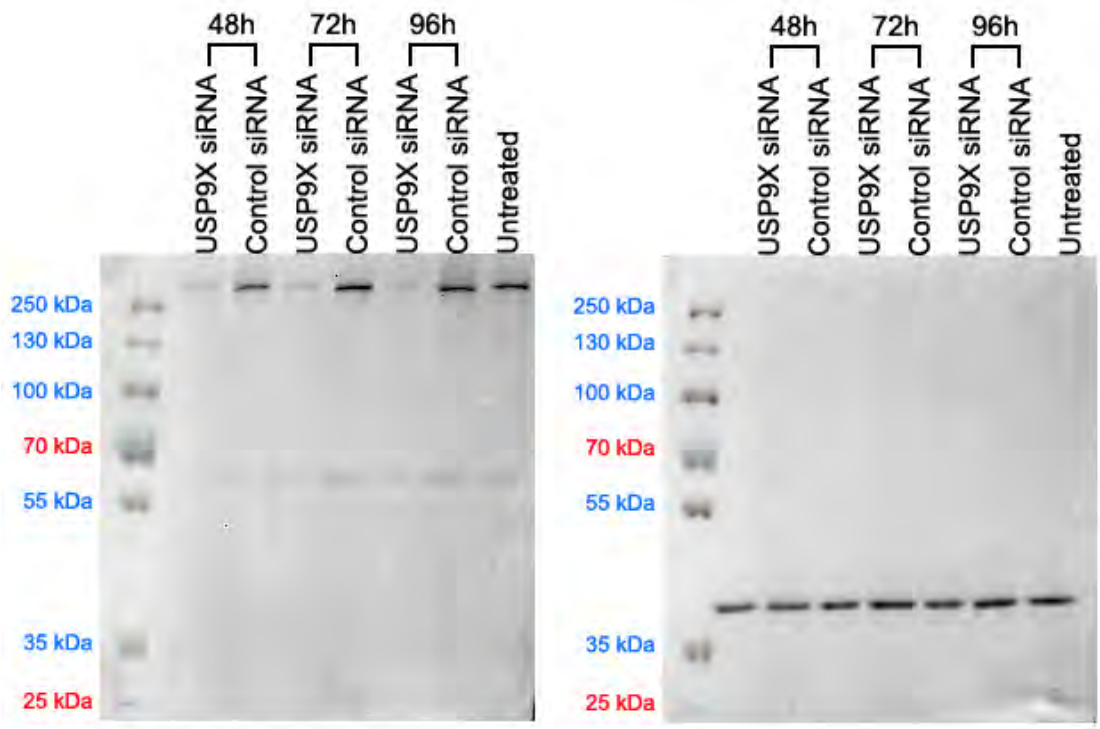

Figure 2. Western blot analysis of USP9x siRNA experiments. USP9x is 290 KDa. The left panel shows the Western blot probed with USP9x antibody and the right panel is probed with GAPDH antibody as a control.

- The results of this study show Usp9x protein decreased at 48, 72 and 96 hours. These results confirmed that (A) Dharmacon reagents (i.e transfection buffer etc) were working and (b) hONS cells are susceptible to siRNA mediated knock-down.

- Results from Western blots using antibody to MAP4K1 indicate that antibody is not working properly since no band of the expected molecular weight is detected. The results from the Western blots probed with antibodies to PTPN1 are shown in Figure 3.

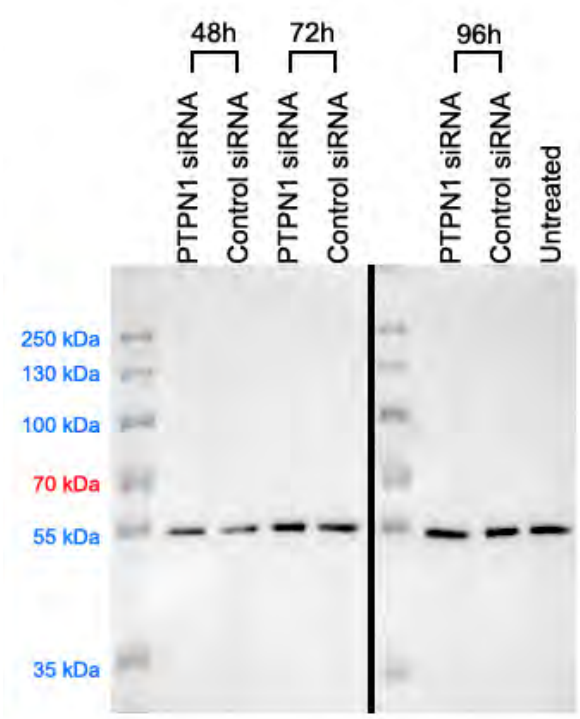

Figure 3. Western blot of PTPN1 siRNA experiment. The results indicate that a protein of the expected size is detected, however the siRNA knockdown did not work.

- Similar experiments are in progress for ZNF160. A summary of our results for the studies in Aim 2 is that we now have antibodies to PTPN1 and ZNF160, which appear to recognize a protein of the expected size. However, Dharmacon siRNAs against PTPN1 have not reduced protein abundance. We are currently testing the siRNAs from Invitrogen / Life Technologies against all three genes of interest.

Objective 3. To identify the signaling pathways affected in PD.

- We used an integrative network based approach to identify dysregulated pathways in PD and type 2 diabetes mellitus (T2DM) based on our earlier studies that indicated that both disease shared disrupted pathways (Santiago and Potashkin, 2013). While these experiments were ongoing, Jose Santiago attended the Genome Access course at Cold Spring Harbor laboratory in July. Based on the information presented in the course, it was clear that we needed to modify the methods we were using. Based on suggestions from the instructors of the course, we initially focused on genes known to be associated with both diseases. Therefore, we retrieved genes associated with PD and T2DM with a genome-wide significance level of $p<10^{-08}$ from the GWAS catalog (http://www.genome.gov/gwastudies/). A random walk algorithm (RWR) with restart was performed using the Gene Prioritization and Evidence Collection (GPEC). We used the weighted and undirected human functional network (FLN) for the analysis. Confirmed genes associated with PD and T2DM obtained, were specified as the training set. The candidate set included neighboring genes within a distance of less or equal than 1. To perform the RWR, we set back-probability to 0.5 and candidate genes were scored and ranked. Biological and functional analysis was performed using the Genemania. Using this method we identified numerous shared susceptibility genes between PD and T2DM including amyloid precursor protein (*APP*) (Santiago and Potashkin, 2013) Published 20-Dec-2013.

- We evaluated the applicability of the network prioritization approach by testing *APP* mRNA as diagnostic biomarkers for PD. Quantification of RNA from whole blood of 192 samples from

two independent clinical trials (PROBE and HBS) revealed that *APP* is upregulated in PD patients compared to healthy controls (Santiago and Potashkin, 2013) Published 20-Dec-2013.

- Biological and functional analysis identified the protein serine-threonine kinase activity, MAPK cascade, activation of the immune response, and insulin receptor and lipid signaling as convergent pathways (Santiago and Potashkin, 2013) Published 20-Dec-2013.

- We used a different genetically and environmentally based bioinformatic network analyses to identify additional mRNA biomarkers for PD. This alternate method may be useful for identifying factors involved with idiopathic disease that have an environmental component to disease etiology and/or development, such as PD. Biological and functional analysis identified nitric oxide biosynthesis, lipid and carbohydrate metabolism, insulin secretion and inflammation as common dysregulated pathways. Superoxide dismutase 2 (*SOD2*) and hepatonuclear factor 4α (*HNF4a*) were identified, tested and validated as blood biomarkers useful for distinguishing PD from healthy controls in these studies. *HNF4a* mRNA significantly correlated with the Hoehn and Yahr scale rating in PD, suggesting its potential use as a progression marker. (Santiago et al., submitted) Submitted 11-Mar-2014.

KEY RESEARCH ACCOMPLISHMENTS

- Developed an integrative bioinformatic network analysis method in order to investigate the extent to which Parkinson's disease (PD) and diabetes are linked at the molecular level, one is genetically focused, the other is environmentally focused to identify factors involved with idiopathic PD.
- Identified three additional risk markers for PD including *APP, SOD2* and *HNF4α.*
- Determined that *HNF4A* mRNA may be a progression marker in blood for PD.
- Identified convergent molecular pathways dysregulated in PD and T2D.
- Published an extensive review on the role of nutrition in PD. The study revealed that a well-balanced diet rich in a vegetables and fruits, omega-3 fatty acids, tea, caffeine, and wine may provide neuroprotection.

REPORTABLE OUTCOMES

1. Poster presentation entitled "Splice variant specific blood biomarkers of Parkinson's disease" at the RNA 2013 meeting, Davos, Switzerland. 14-Jun-2013.
2. Published a paper entitled "Specific splice variants are associated with Parkinson's disease" (Santiago et al., 2013), 12-Sep-2013.
    - Validated 7 blood risk markers in a separate cohort of study participants (HBS study) that may be used to distinguish Parkinson's patients (PD) from healthy controls (HC).
3. Seminar presentation entitled "Shared dysregulated pathways lead to Parkinson's disease and diabetes" at the Grand Challenges in Parkinson's Disease: the role of inflammation meeting, Van Andel Institute, Grand Rapids, MI. 18-Sep-2013.
4. Published a paper entitled "Integrative network analysis unveils convergent molecular pathways in Parkinson's disease and diabetes" (Santiago and Potashkin, 2013). Submitted 24-Sep-2013. Accepted 20-Dec-2013.
    - Identified amyloid precursor protein (*APP*) mRNA as a blood risk marker that may be used to distinguish PD from HC.
    - Developed a bioinformatic approach for identifying genetic factors involved in PD and diabetes.
    - Revealed convergent molecular pathways that are dysregulated in PD and type 2 diabetes (T2D). Manuscript attached to report.
5. Published a paper entitled "The Emerging Role of Nutrition in Parkinson's Disease" to Frontiers in Aging Neuroscience (Seidl et al., 2014). Submitted 5-Nov-2013. Revision submitted 16-Dec-2-13.
    - The goal of our studies is to identify biomarkers predictive of PD. If we are successful, it would be beneficial to be able to provide PD patients with information with regards to lifestyle changes that may be helpful with managing the disease. With this in mind we

reviewed the literature to determine if diet may play a role in development or management of PD. Our research lead to the following conclusions.

- A poor diet may lead to increased oxidative stress, which could impede the antioxidant defense system.
- In contrast, a well-balanced diet rich in a variety of foods, including numerous servings of vegetables and fruits (especially those containing nicotine) and moderate amounts of omega-3 fatty acids, tea, caffeine, and wine may provide neuroprotection.
- In spite of promising effectiveness of these nutrients in PD, we lack definitive evidence-based answers as a result of limited large prospective randomized controlled studies designed to address these issues.
- This article will be featured on the website Value-Based Care in Neurology.

6. Seminar presentation by Judy Potashkin entitled "A network approach to diagnostic biomarkers in neurodegenerative diseases" at the Neuroscience retreat Rosalind Franklin U., North Chicago, IL. 5-Dec-2013.
7. Published a paper entitled "System-based approaches to decode the molecular links in Parkinson's disease and diabetes" to *Neurobiology of Disease* for a special issue on Metabolic Disorders and Neurodegeneration. 14-Jan-2014. Submitted 14-Jan-2014. Revision submitted 24-Mar-2014. Accepted 28-Mar-2014.
    - In this review, we discuss the current experimental approaches to study the association between PD and T2DM and the potential therapeutic targets these system models have elucidated. The models discuss include the network analysis used in our recent research to identify biomarkers of PD.
8. Submitted a manuscript entitled **"**Network analysis identifies *HNF4A* and *SOD2* mRNAs as biomarkers for Parkinson's Disease" to *Neurobiology of Aging*. Submitted 11-Mar-2014, currently under review.
    - In this study we integrate data from public databases and perform network analysis to study the linkage between PD and T2DM. In order to translate these results into a clinically relevant tool for disease diagnosis, we tested highly ranked genes, *HNF4A* and *SOD2* on RNA prepared from whole blood. The results indicate that both transcripts are biomarkers for early stage PD.
    - The relative abundance of *HNF4A* mRNA significantly correlated with the Hoehn and Yahr scale rating in PD, suggesting its potential use as a progression marker.
    - Biological and functional analysis identified nitric oxide biosynthesis, lipid and carbohydrate metabolism, insulin secretion and inflammation as common dysregulated pathways.
    - Our results provide evidence that PD and T2DM are strongly linked at the molecular level and that analysis of shared molecular networks provide a means to identify biologically meaningful biomarkers including potential markers of disease progression.

CONCLUSION

Currently we have identified 17 mRNA biomarkers (13 from an earlier study and 4 during the past year) in blood that may be used to distinguish early stage PD patients from healthy and neurological controls. The 4 recently identified markers were identified using network analysis. We will continue to refine our network analysis since it has proved to be a very productive approach for identifying biomarkers and pathways dysregulated in PD. Further testing on the markers using the samples from the PARS study will determine whether the markers are useful for identifying individuals at risk for PD.

One of the recent markers we identified, *HNF4α* mRNA, significantly correlated with the Hoehn and Yahr scale rating in PD, suggesting its potential use as a progression marker. Further studies on *HNF4α* mRNA are needed to determine if it indeed is a progression marker.

In order to test dysregulated pathways identified by network analysis we are presently using hONS. We are currently trouble-shooting problems with the antibodies and siRNAs. We

expect that some of these problems may be resolved by buying supplies from a different distributor.

So what do these results mean for the PD patient? Since diagnosis of PD currently relies on assessment of motor symptoms and misdiagnosis occurs with other parkinsonian disorders, the availability of specific and sensitive molecular markers would be useful in the clinic for reducing diagnostic errors. The 10 biomarkers that have been validated in two independent clinical trials (Santiago et al., 2013; Santiago and Potashkin, 2013; Santiago and Potashkin, submitted) may be developed into a marketable PCR assay that can be used in the clinics. In addition, network analysis has defined some of the pathways that are dysregulated in PD. This information may now be used to identify potential therapeutic targets of PD. And last, but not least, identification of nutrients that are potentially neuroprotective for PD provides clinicians with information that may be used in counseling their patients (Seidl et al, 2014). In this regard, this study will be featured on the website Value-Based Care in Neurology.

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APPENDICES

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Potashkin curriculum vitae

Specific Splice Variants Are Associated With Parkinson's Disease

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ABSTRACT

Background: Diagnosis of Parkinson's disease (PD) currently relies on assessment of motor symptoms. Recently, sensitive, specific, and readily available splice variant–specific biomarkers were identified in peripheral blood from participants in the Diagnostic and Prognostic Biomarkers in Parkinson Disease study.

Methods: Here we test for an association between candidate splice variant biomarkers and PD in blood of an independent population of cases and controls nested in the Harvard NeuroDiscovery Center Biomarker Study.

Results: Expression of 7 out of 13 candidate biomarkers was dysregulated in whole cellular blood of patients with PD.

Conclusions: These results support the view that differential expression of a subset of splice-variant markers in blood is associated with PD. Further evaluation in untreated, de novo patients and at-risk subjects is warranted.

Key Words: Parkinson's disease; biomarker; neurodegeneration; splicing; gene expression

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Diagnosis of PD has classically relied on motor symptoms including resting tremor, rigidity, bradykinesia, and postural instability. The availability of accessible diagnostic biomarkers would be beneficial for identifying presymptomatic patients and for following the progression of the disease. In this regard, several studies have examined gene expression profiling in blood to identify molecular signatures associated with PD.[1–3] Likewise, we previously identified 13 splice-variant biomarkers in whole blood that could be used to distinguish PD patients from healthy and neurological controls.[4] In order to confirm the association between biomarkers levels in blood and PD we tested them in an independent cross-sectional case-control study nested in the Harvard NeuroDiscovery Center Biomarker Study (HBS). We confirm associations between 7 of the candidate biomarkers and PD in the HBS population.

Subjects and Methods

Study Populations

The Institutional Review Boards of Rosalind Franklin University of Medicine and Science and Brigham and Women's Hospital approved the study protocol. Written informed consent was received from all participants. 96 individuals including 50 PD patients (mean Hoehn & Yahr scale 2; Table 1) and 46 healthy HC age-matched controls were enrolled in the HBS. Patient and control recruitment, clinical assessments, and biobanking in the HBS population have been reported in part elsewhere[5] and online (http://www.neurodiscovery.harvard.edu/research/biomarkers.html).

RNA Preparation and Gene Expression Analysis

Blood was collected and prepared as described using the PAXgene Blood RNA system (Qiagen, Valencia, CA, USA).[1] Samples with RNA integrity values >7.0 (indicating excellent RNA integrity) and ratio of absorbances at 260/280 nm between 1.7 and 2.4 were used in the current study. The High Capacity RNA transcription kit (Applied Biosystems, Foster City, CA, USA) was used to reverse transcribe 1 μg of total RNA according to the manufacturer's protocol. The primers and amplification conditions have been published.[4]

A stepwise multivariate discriminant linear regression was performed on the expression data adjusting for covariates including body mass index (BMI), sex, and age, and a correlation analysis was used to determine if individual variables correlate with each other using Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). Network analysis was done using the GeneMania

TABLE 1. Clinical characteristics of study participants

Characteristics	Disease status		
	PD	HC	*P*
Participants, n	50	46	>0.5
Age at enrollment, y	63.12 ± 8.96	64.28 ± 10.42	>0.5
Age of onset, y	58.75 ± 10.17	N/A	
Male, n	31	26	>0.5
Female, n	19	20	>0.5
BMI (mean ± SD)[a]	N (16): 22.81 ± 1.54	N (19): 22.26 ± 2.09	>0.5
	OW (22): 27.08 ± 1.35	OW (12): 26.92 ± 1.42	0.0001
	OB (12): 35.65 ± 3.43	OB (15): 33.14 ± 2.98	>0.5
	OW+OB (34); 30.36 ± 4.82	OW+OB (27): 29.77 ± 3.86	0.01
Hypertension, n	18	15	>0.5
Diabetes, n	5	5	>0.5
Hoehn & Yahr	1.97 ± 0.62	N/A	

Values are mean ± SD.
[a]BMI was defined by standard measures as N = 18.5–24.9, OW = 25.0–29.9, and OB = ≥30. Values in parentheses are numbers of participants at each BMI level.
PD, Parkinson's disease; HC, healthy control; BMI, body mass index; N, normal; OW, overweight; OB, obese.

prediction server.[6] A 2-tailed Student *t* test was used to estimate the significance between PD and controls (GraphPad Software, La Jolla, CA, USA).

Results

The mean age of onset and Hoehn & Yahr rating of the PD patients in this study was 58.75 ± 10.17 and 1.97 ± 0.62, respectively (Table 1). Relative mRNA expression levels revealed a significant upregulation of expression of splice variants of c5orf4 ($P = 0.006$), copz1 ($P = 0.00003$), eftud2 ($P = 0.0001$), macf1 ($P = 0.03$), prg3 ($P = 0.002$), wls ($P = 0.006$), and znf160 ($P = 0.00008$) in whole blood of PD patients compared to HC in the univariate analysis (Fig. 1A). The direction of the gene expression change of each confirmed splice variant is consistent with that previous reported.[4] Six splice variants, including slc14a1-s, slc14a1-l, map4k1, mpp1, znf134, and pkm2, did not show significant association in this study population ($P > 0.05$). A priori power analysis was carried out using the results from the previous study[1] to demonstrate that a fold change of 1.5 or higher could be determined with a 90% power using 40 samples per group with a significance level of 0.05. Correlation analysis revealed that none of the variables correlate with each other, with correlation values ranging from 0.27 to 0.52. Regression analysis revealed that expression of each biomarker was independent from BMI ($P = 0.55$), age at enrollment ($P = 0.50$), age of onset ($P = 0.30$), and sex ($P = 0.48$). Correlation of biomarker expression with drug dose was not evaluated since most of the patients with PD were medicated with several drugs and the number of untreated patients was to small to reliably detect a significant change.

In order to build a model with the highest predictive accuracy, a stepwise multivariate linear discriminant regression (LDA) was performed on the gene expression data, adjusting for covariates. This type of analysis evaluates the discriminant power of each interrogated variable in each step, thus building a prediction model by progressively adding the variables with the most significant individual *P* value ($P \leq 0.05$) at each step. Based on this analysis, a 7-gene panel was found to discriminate PD patients from controls. The resulting canonical discriminant equation is $D_{PD} = 0.170 * X_{copz1} + 0.130 * X_{c5orf4} + 0.106 * X_{znf160} - 0.288 * X_{eftud2} + 0.081 * X_{wls} + 0.070 * X_{prg3} - 0.133 * X_{macf1} - 1.28$, where D_{PD} is the discriminant score value (raw canonical coefficients) and Xi is the mRNA expression level of each biomarker. The 4 most significant predictors were znf160 (0.75), copz1 (0.70), c5orf4 (0.45), and wls (0.38) (standardized coefficients). Although the number of overweight (BMI > 25) and obese (BMI > 30) participants was significantly higher in PD than HC ($P < 0.01$), it had no impact on the prediction model ($P = 0.90$). Other covariates including sex and age were also removed from the prediction model by stepwise analysis (sex, $P = 0.88$; age, $P = 0.65$).

LDA was also used to determine the predictive accuracy of the biosignature to discriminate between PD patients and HC. The average discriminant score for PD patients and HC controls was −0.92 ± 0.17 and 1.0 ± 0.09, respectively. Patients with discriminant scores below 0.20 ($D \leq 0.20$) were classified as PD and patients above the cutoff value were classified as HC. Based on this analysis, PD patients were identified with 78% sensitivity and 90% specificity and HC controls with 94% sensitivity and 93% specificity.

Network analysis indicated that 6 of the biomarkers were connected and the most significant canonical pathways dysregulated in PD were the Golgi vesicle transport and RNA processing (Fig. 1B).

Discussion

Environmental factors play a key role in regulating many steps of gene expression including alternative

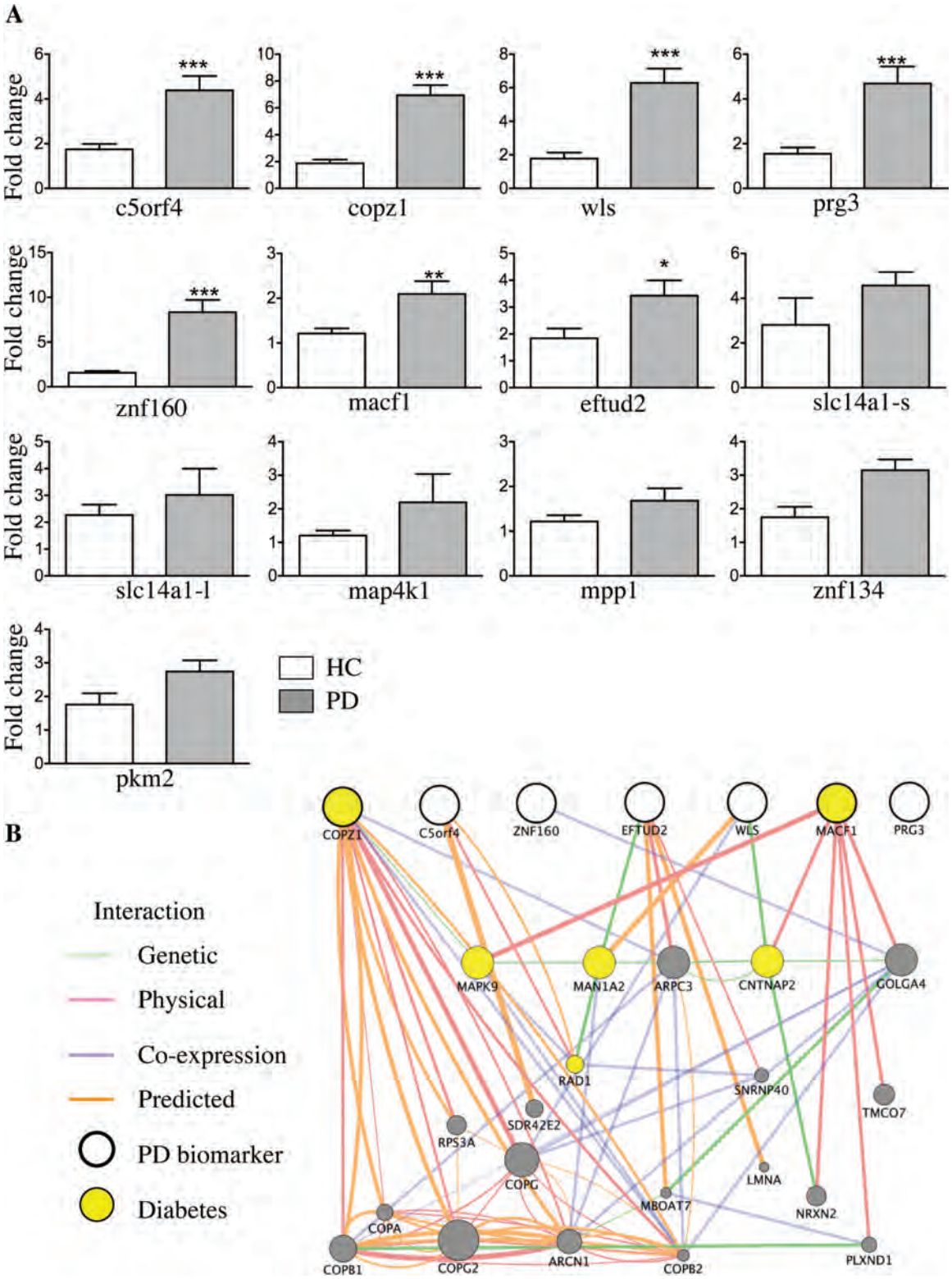

FIG. 1. A: Relative mRNA expression levels of biomarkers in samples obtained from participants of the HBS study. Expression levels at the enrollment visit are shown. Fold change of each splice variant was calculated using gapdh as a reference gene and expression levels in the HC as a calibrator. *P* values (**P* = 0.01; ***P* < 0.001; ****P* < 0.0001). HC indicates healthy controls and PD indicates patients with Parkinson's disease; n = 50 PD patients, n = 46 HCs. Error bars represent standard error. **B:** Network analysis of the biomarkers. Candidate PD biomarkers are shown in bold-faced white circles. Genes associated with diabetes are displayed in yellow. Interaction is shown by color-coded lines (green = genetic; pink = physical; purple = coexpression; orange = predicted).

splicing. The consequence of regulated splicing is the production of several splice variants from a single pre-messenger ribonucleic acid (pre-mRNA).

Because of the rapid response of the splicing machinery to environmental factors, which play a key role in the development of PD, we tested the hypothesis that PD patients may be identified using splice variant–specific biomarkers. Here we replicated an association between expression levels of 7 splice variants previously identified.[1] and PD in a new case-control study. Six of the splice variants showed no statistically significant association with PD in this cohort. However, these markers may be useful for distinguishing PD patients from other atypical parkinsonian disorders.[1] Network analysis revealed that macf1 and copz1, both genes associated with diabetes,[7] interact with the mitogen-activated protein kinase 9 (mapk9) (Fig. 1B). Interestingly, disruption of the mapk9 gene, which encodes the cJun N-terminal kinase 2 (JNK2), reduced insulitis, hyperglycemia, and disease progression in diabetic mice.[8] In addition, JNK2 expression is associated with insulin resistance and inflammation and plays a key role in obesity.[9] Another central node within the network was mannosidase, alpha, class 1A, member 2 (man1a2). Recent evidence suggests that man1a2 is targeted by the peroxisome proliferator-activated receptor (PPAR-γ) in a novel anti-inflammatory mechanism in vascular endothelial cells[10] (Fig. 1B). This finding is interesting in light of the fact that PPAR-γ coactivators and antidiabetic drugs targeting PPARs are neuroprotective in models of PD.[11,12]

Given the links between PD and diabetes discussed, future research directed at understanding common dysregulated pathways may enable novel therapeutic strategies for PD.[7] Although these biomarkers have been replicated in an independent cohort of patients, the results from this cross-sectional study may be vulnerable to bias from unanticipated confounds. For example, differences in blood counts and Parkinson's medications may bias gene expression results. Thus, evaluation of these biomarkers in patients not treated with PD medications and in a large well-characterized prospective study will be important to determine the clinical utility of these findings. Determining whether these markers are useful for distinguishing individuals at risk for PD, for progression of PD, and/or for distinguishing subcategories of PD patients will be important for future research. ■

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Appendix

Harvard NeuroDiscovery Center Biomarker Study: Co-Directors: Harvard NeuroDiscovery Center: Clemens R. Scherzer, Bradley T. Hyman, Adrian J. Ivinson; Investigators and Study Coordinators: Harvard NeuroDiscovery Center: Ana Trisini-Lipsanopoulos, Kaltra Dhima, Stephen Bayer, Kaitlin C. Lockhart; Brigham and Women's Hospital: Lewis R. Sudarsky, Michael T. Hayes, Reisa Sperling; Massachusetts General Hospital: John H. Growdon, Michael A. Schwarzschild, Albert Y. Hung, Alice W. Flaherty, Deborah Blacker, Anne-Marie Wills, U. Shivraj Sohur, Vivek K. Unni, Nicte I. Mejia, Anand Viswanathan, Stephen N. Gomperts, Vikram Khurana, Mark W. Albers, Rebecca K. Rudel; University of Ottawa: Michael G. Schlossmacher; Scientific Advisory Board: Massachusetts General Hospital: John H. Growdon, Brigham and Women's Hospital: Lewis R. Sudarsky, Dennis J. Selkoe, Reisa Sperling; Harvard School of Public Health: Alberto Ascherio; Data Coordination: Harvard NeuroDiscovery Center: Thomas Yi, Massachusetts General Hospital: Joseph J. Locascio; Biobank Management Staff: Harvard NeuroDiscovery Center: Zhixiang Liao, Ashley N. Hoesing, Karen Duong, Sarah Roderick.

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Integrative Network Analysis Unveils Convergent Molecular Pathways in Parkinson's Disease and Diabetes

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Abstract

Background: Shared dysregulated pathways may contribute to Parkinson's disease and type 2 diabetes, chronic diseases that afflict millions of people worldwide. Despite the evidence provided by epidemiological and gene profiling studies, the molecular and functional networks implicated in both diseases, have not been fully explored. In this study, we used an integrated network approach to investigate the extent to which Parkinson's disease and type 2 diabetes are linked at the molecular level.

Methods and Findings: Using a random walk algorithm within the human functional linkage network we identified a molecular cluster of 478 neighboring genes closely associated with confirmed Parkinson's disease and type 2 diabetes genes. Biological and functional analysis identified the protein serine-threonine kinase activity, MAPK cascade, activation of the immune response, and insulin receptor and lipid signaling as convergent pathways. Integration of results from microarrays studies identified a blood signature comprising seven genes whose expression is dysregulated in Parkinson's disease and type 2 diabetes. Among this group of genes, is the amyloid precursor protein (*APP*), previously associated with neurodegeneration and insulin regulation. Quantification of RNA from whole blood of 192 samples from two independent clinical trials, the Harvard Biomarker Study (HBS) and the Prognostic Biomarker Study (PROBE), revealed that expression of *APP* is significantly upregulated in Parkinson's disease patients compared to healthy controls. Assessment of biomarker performance revealed that expression of *APP* could distinguish Parkinson's disease from healthy individuals with a diagnostic accuracy of 80% in both cohorts of patients.

Conclusions: These results provide the first evidence that Parkinson's disease and diabetes are strongly linked at the molecular level and that shared molecular networks provide an additional source for identifying highly sensitive biomarkers. Further, these results suggest for the first time that increased expression of *APP* in blood may modulate the neurodegenerative phenotype in type 2 diabetes patients.

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Introduction

Parkinson's disease and type 2 diabetes are among the most prevalent diseases affecting the aging population. Recent findings have revealed convergent molecular and biological pathways that link both diseases. Mitochondrial dysfunction, endoplasmic reticulum stress, inflammation and alterations in glucose metabolism are disrupted in both diseases [1]. Exposure to environmental factors and genetic susceptibility are thought to be involved in the etiology of both diseases. Accordingly, most cases of Parkinson's disease and type 2 diabetes are considered sporadic with 5–10% attributed to known genetic factors. Several shared genetic connections between diabetes and Parkinson's disease have recently been identified. For example, regulation of expression of *PINK1*, previously associated with Parkinson's disease [2], is altered in skeletal muscle of type 2 diabetes patients [3]. Likewise, DJ-1, an antioxidant protein with reduced expression in Parkinson's disease is also reduced in pancreatic islets of type 2 diabetes patients and increases during aging under non-diabetic conditions [4]. To date, there is no modifying agent or preventive treatment available but commonly prescribed drugs to treat diabetics have shown promise in Parkinson's disease clinical trials [5,6]. Neuroprotection conferred by these drugs is attributed to the targeting of the inflammatory pathways. In addition to inflammation, impaired insulin signaling and glucose metabolism, hallmarks of diabetes, may play a role in the development and progression of Parkinson's disease, therefore understanding the molecular framework that links both diseases is expected to facilitate the development of novel therapeutic strategies.

High-throughput methods have successfully identified thousands of genetic associations with Parkinson's disease and type 2 diabetes. However, the large amount of data is difficult to integrate and it is often problematic to interpret the underlying functional disease mechanism based on the annotation of a single gene.

Complex diseases such as Parkinson's disease and type 2 diabetes are affected by many genes that may act synergistically to contribute to disease development perhaps by participating in common biological pathways. Network biology has emerged as a powerful tool for the interpretation and integration of genomic data to understand disease-disease and gene-disease associations [7–11]. In this context, integrated network-based approaches have been used to identify pathways and susceptibility genes associated with Parkinson's disease and type 2 diabetes. For example, using an integrative systems biology approach, axon guidance, focal adhesion, and calcium signaling were identified among the most significant pathways in Parkinson's disease [12]. Likewise, using a network approach a set of genes associated with insulin signaling and nuclear receptors were identified in type 2 diabetes models [13]. In addition, analysis of metabolite-protein networks identified biomarkers for pre-diabetes [14].

Here we employ an integrated network approach to dissect the molecular networks and dysregulated pathways shared between Parkinson's disease and type 2 diabetes. Our network approach utilizes a random walk based algorithm (RWR) to quantitatively prioritize genes according to their topological distance and functional relatedness with known disease genes in the functional linkage network (FLN) [15]. The use of the FLN as a platform to rank potential disease-related genes is based on the premise that a group of genes known to contribute to a particular disease phenotype are usually functionally related. The weight of each link between a pair of genes represents the likelihood that the linked genes share common biological processes. In addition, we integrate data from previous microarray studies to identify a whole blood signature characteristic of Parkinson's disease and type 2 diabetes. In order to translate these results into a clinically relevant tool for disease diagnosis, we evaluate the expression of *APP* in blood of Parkinson's disease patients in samples from two independent clinical trials. In this study we provide evidence that Parkinson's disease and type 2 diabetes are highly interconnected at the molecular level. Further, this study supports the idea that complex diseases like Parkinson's disease and type 2 diabetes may result as a consequence of perturbations in shared molecular networks.

Methods

Genes associated with Parkinson's disease and type 2 diabetes were retrieved from the GWAS catalog (http://www.genome.gov/gwastudies/). Genes with a genome-wide significance level of $p<10^{-08}$ were included in this study. A random walk algorithm with restart (RWR) was performed using Gene Prioritization and Evidence Collection (GPEC), a Cytoscape 2.8.3 plugin [16]. We used the weighted and undirected human FLN for this analysis [17]. Confirmed genes associated with Parkinson's disease and type 2 diabetes obtained from the GWAS catalog, were specified as the training set (Tables S1 and S2). The candidate set included neighboring genes within a topological distance of less or equal than 1 in the FLN. The RWR algorithm is formally defined elsewhere [15]. Briefly, the RWR moves from a seed node to a randomly immediate neighboring node or returns to the start node with a probability α at each step [15]. To perform the RWR, we set the restart probability α to 0.5 and candidate genes were scored and ranked. RWR scores for prioritized genes are listed in Table 1 and Table S3. Biological and functional analysis was performed using the Genemania plugin [18].

Table 1. RWR scores for the top 20 ranked genes.

Rank	Gene	Score
1	CD63	4.45E-03
2	CDK1	4.26E-03
3	USHBP1	2.18E-03
4	RAF1	1.43E-03
5	PKN1	1.21E-03
6	MAPK1	9.54E-04
7	RHOA	8.98E-04
8	CREBBP	8.71E-04
9	COPB1	7.83E-04
10	AKT1	7.68E-04
11	ARF1	7.68E-04
12	BRAF	7.59E-04
13	RALGDS	7.01E-04
14	ARF3	6.99E-04
15	APP	6.97E-04
16	POU4F1	6.90E-04
17	ROCK2	6.76E-04
18	MAPK3	6.75E-04
19	PRKCA	6.54E-04
20	ROCK1	6.48E-04

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Ethics statement and PROBE and HBS study participants information

The Institutional Review Boards of Rosalind Franklin University of Medicine and Science approved the study protocol. Written informed consent was received from all participants. 96 individuals including 50 Parkinson's disease patients (mean Hoehn and Yahr scale 2, Table 2) and 46 healthy age-matched controls were enrolled in the HBS. Details of patient and controls recruitment, clinical assessments, and biobanking in the HBS study population have been reported in part elsewhere [19] and http://www.neurodiscovery.harvard.edu/research/biomarkers.html. As an independent replication set, we used 51 Parkinson's disease patients (mean Hoehn and Yahr scale of 2) and 45 healthy age-matched controls enrolled in the PROBE Study (#NCT00653783). Clinical diagnosis of Parkinson's disease was based on the United Kingdom Parkinson's Disease Society Brain Bank criteria [20]. Healthy controls had no history of neurological disease and a Mini-Mental State Examination (MMSE) test score higher than 27. Details of patient and controls recruitment, clinical assessment, inclusion and exclusion criteria have been reported in part elsewhere [21]. Clinical description of study participants is listed in Table 2.

RNA isolation and real time polymerase chain reactions

Blood was collected and prepared as described using the PAXgene Blood RNA system (Qiagen,Valencia, CA) [22]. Samples with RNA integrity values >7.0 and a ratio of absorbances at 260/280 nm between 1.7 and 2.4 were used in the current study. Primer Express software (Applied Biosystems, Foster City, CA) was used to design the primers. Primer sequences used in qPCR assays are as follows: app; forward: 5′-TTTTCTAGAGCCTCAGCGTCCTA-3′; reverse: 5′- CCCTGGGCTTCGTGAACA-3′, gapdh; forward: 5′-CAACGGATT-

Table 2. Clinical characteristics of HBS and PROBE study participants.

HBS			
Disease status	**PD**	**HC**	**p-value**
Number	50	46	>0.5
Age at enrollment (Mean ± SD)	63.12±8.96	64.28±10.42	>0.5
Age of onset (Mean ± SD)	58.75±10.17	N/A	>0.5
Male	31	26	>0.5
Female	19	20	>0.5
BMI (Mean ± SD)	N (16); 22.81±1.54	N (19); 22.26±2.09	>0.5
	OW (22); 27.08±1.35	OW (12); 26.92±1.42	0.0001
	OB (12); 35.65±3.43	OB (15); 33.14±2.98	>0.5
	OW+ OB (34); 30.36±4.82	OW + OB (27); 29.77±3.86	0.01
Hypertension	18	15	>0.5
Diabetes	5	5	>0.5
Hoehn & Yahr (Mean ± SD)	1.97±0.62	N/A	>0.5
PROBE			
Number	51	45	>0.5
Age at enrollment (Mean ± SD)	63.16±6.42	65.12±8.60	>0.5
Male	29	24	>0.5
Female	22	21	>0.5
Diabetes	0	1	>0.5
Hoehn & Yahr (Mean ± SD)	2±0.28	N/A	>0.5

BMI is body mass index, N is normal, OW is overweight and OB is obese. BMI was defined by standard measures as normal (N) = 18.5–24.9, overweight (OW) = 25–29.9 and obese (OB) = 30 or greater.

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TGGTCGTATTGG-3′; reverse: 5′-TGATGGCAACAATATC-CACTTTACC-3′. The High Capacity RNA transcription kit (Applied Biosystems, Foster City, CA) was used to reverse transcribe 1 µg of total RNA according to the manufacturer's protocol. The DNA engine Opticon 2 Analyzer (Bio-Rad Life Sciences, Hercules, CA) was used for the qPCR reactions. Each 25 µl reaction contained Power SYBR and primers at a concentration of 5 µM. The amplification conditions used are as follows: denature at 95°C for 15 sec, annealing at 57°C for 1 min, extension at 75°C for 45 sec for 45 cycles of amplification. Following the PCR reaction a melting curve analysis was run to confirm that a single product was amplified. PCR products were also run on 1.5% agarose gels to verify specificity. Gapdh was used as a reference gene. Samples were loaded in triplicate. No cDNA template, PD and HC positive controls were run in every experiment. Amplification efficiencies were higher than 90% for each primer set. Expression data was analyzed using the ΔΔCt method.

Statistical analysis

All analyses were performed with Prism4.0 (Graphpad, La Jolla, CA) and Statistica 8.0 (StatSoft, Tulsa, OK, USA). A student t-test (two-tailed) followed by a Tukey-Kramer post-hoc analysis was used to estimate the significance between PD cases and controls. Linear regression was performed on the expression data adjusting for covariates including, sex and age and BMI in the HBS cohort. Correlation analysis was used to determine if individual variables correlate with each other. Microarray data was analyzed using a Benjamini and Hochberg analysis with a FDR = 0.05. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic accuracy of the biomarker. A p-value less than 0.05 was considered statistically significant.

Results

Shared molecular network in Parkinson's disease and type 2 diabetes

In order to investigate the extent to which Parkinson's disease and type 2 diabetes are linked at the molecular level, we performed a RWR algorithm within the human FLN to identify genes associated with both diseases (Figure 1). Genetic associations that confer a risk to Parkinson's disease and type 2 diabetes were retrieved from the GWAS catalog. Only genes with a GWAS significance level of $P<10^{-8}$ were included in this study. A total of 23 genetic loci associated with Parkinson's disease risk were identified in the FLN and specified as training genes (Figure 2A, Table S1). Our test set consisted of neighboring genes with topological distance to the training genes of less than or equal to 1 (LD ≤1). A total of 886 genes were functionally linked to confirmed Parkinson's disease genes. In parallel, using 43 genes associated to type 2 diabetes as training genes, we identified a set of 1,705 neighboring genes (Figure 2A, Table S2). Venn diagram analysis revealed that Parkinson's disease and type 2 diabetes shared 478 neighbors within the FLN (Figure 2A). The top 20 genes prioritized by the RWR are listed in Table 1 and the top 200 ranked genes are listed in Table S3.

Biological and functional analysis of the shared cluster of genes identified pathways associated to the protein serine-threonine kinase activity ($p<10^{-95}$), nerve growth factor receptor signaling ($p<10^{-40}$), immune response signaling ($p<10^{-17}$), MAPK

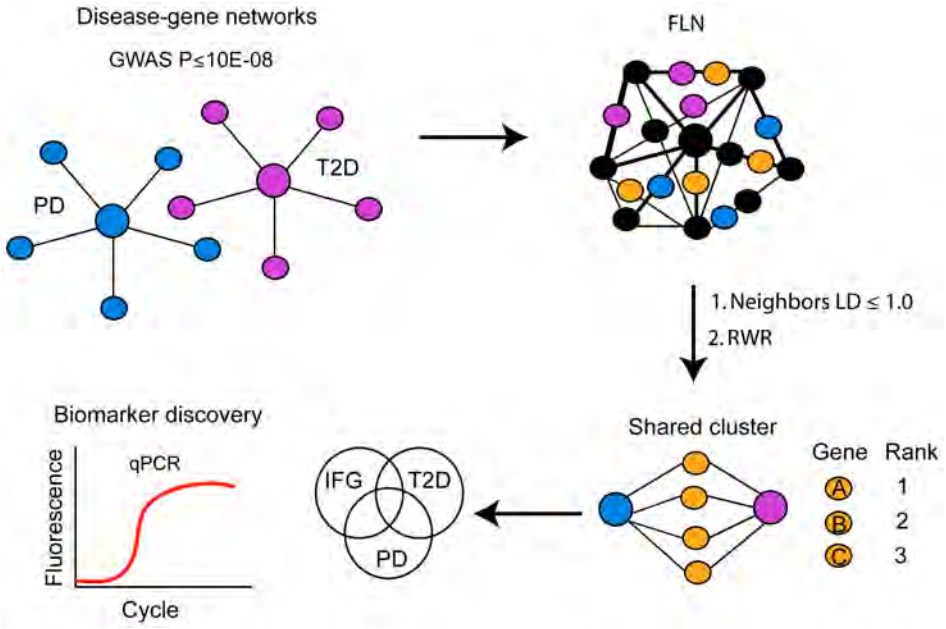

Figure 1. Integrative network approach. Genes with a genome-wide significance of $P<10^{-08}$ or less associated with Parkinson's disease (displayed in blue) and type 2 diabetes (displayed in purple) were included in this study and specified as training genes. A random walk algorithm within the functional linkage network (displayed in gray) was performed to identify candidates genes with a linkage distance (LD) to the training genes of less than or equal to 1 within the FLN. Candidate genes (displayed in orange) were ranked and scored according to their closeness with training genes. Data from microarrays studies in blood of Parkinson's disease, pre-diabetes and Type 2 diabetes patients was analyzed to identify genes dysregulated in both diseases. Quantitative PCR assays were used to validate a potential biomarker in blood of Parkinson's disease patients. Networks were visualized using Cytoscape 2.8.3. PD = Parkinson's disease, IFG = pre-diabetes and T2D = type 2 diabetes.
doi:10.1371/journal.pone.0083940.g001

cascade ($p<10^{-15}$), lipid signaling ($p<10^{-11}$), response to insulin stimulus ($p<10^{-10}$), and insulin receptor signaling ($p<10^{-10}$).

Inspection of network topology revealed interesting genetic interactions among well-characterized genes associated with Parkinson's disease and type 2 diabetes. As shown in Figure 2A, multiple type 2 diabetes genetic risk loci are interrelated with Parkinson's disease susceptibility genes throughout the FLN. For example, *APP* interacts with susceptibility genes to type 2 diabetes (*LAMA1* and *IDE*) and genes associated with Parkinson's disease risk including *SNCA* and *MAPT* (Figure 2B).

A blood signature of Parkinson's disease and type 2 diabetes

Impaired insulin signaling and glucose intolerance, hallmarks of diabetes, are implicated in Parkinson's disease [1,23]. From a system biology perspective, altered expression of genes in peripheral blood may reflect systemic changes observed in both diseases thus providing a better platform to identify disease-specific biomarkers. We interrogated multiple gene expression data sets from independent microarrays studies that used RNA prepared from peripheral whole blood of patients with type 2 diabetes and Parkinson's disease. First, we re-analyzed the study GSE26168 in which changes in mRNA were measured in blood of healthy, impaired fasting glucose, commonly known as pre-diabetes and type 2 diabetes patients. Pair-wise comparisons were performed for each group using a Benjamini and Hochberg analysis with a false discovery rate (FDR) of 0.05 to correct for the occurrence of false positives [24]. In parallel, we re-analyzed microarray data from two previously published studies that compared RNA from whole blood of Parkinson's disease patients compared to healthy individuals (GEO accession numbers: GSE34287, GSE6613). Integration of these microarray studies identified a blood signature of seven transcripts including app, bcl2l1, chpt1, gpr97, ppm1a, and srrm2, common to pre-diabetes, type 2 diabetes, and Parkinson's disease (Figure 3A and B). Only app and gpr97 are upregulated in all groups (Figure 3B). The list of significant genes, fold changes and p-values are listed in Table S4.

We next sought to investigate whether any of the 7 mRNAs were functionally linked to confirmed Parkinson's disease genes in the FLN. Venn diagram analysis identified app as common in both groups (Figure 3C). Interestingly, app mRNA expression was upregulated in pre-diabetes (fold change 1.47, $p<0.05$)[25] and in Parkinson's disease (2.24, $p<0.05$)[21] (Figure 3D).

Biomarker discovery and validation

Given the numerous molecular links between Parkinson's disease and type 2 diabetes identified in the FLN and microarray studies, we sought to translate these results into a more relevant tool with clinical applicability. Taking into consideration the results generated by integrated network analysis, we evaluated *APP* as a potential biomarker for Parkinson's disease. Relative mRNA levels of *APP* were measured in whole blood of Parkinson's disease patients compared to healthy individuals from samples obtained from two independent clinical trials, the Harvard Biomarker Study (HBS) and the Prognostic Biomarker Study (PROBE). Description of the study participants is listed in Table 2. Gene expression analysis by qPCR revealed that *APP* is significantly upregulated in blood of Parkinson's disease patients compared to healthy controls in the HBS cohort (Mean ± SEM; 4.96±0.98, $p=0.01$) and PROBE study (Mean ± SEM; 5.11±1.0, $p=0.01$) (Figure 4 A and B). Correlation analysis demonstrated that expression of *APP* was independent of other covariates including age ($R=0.01$, $p>0.05$), sex ($R=0.01$, $p>0.05$), Hoehn and Yahr scale ($R=0.09$, $p>0.05$) in both cohorts of patients and BMI ($R=0.01$, $p>0.05$) in

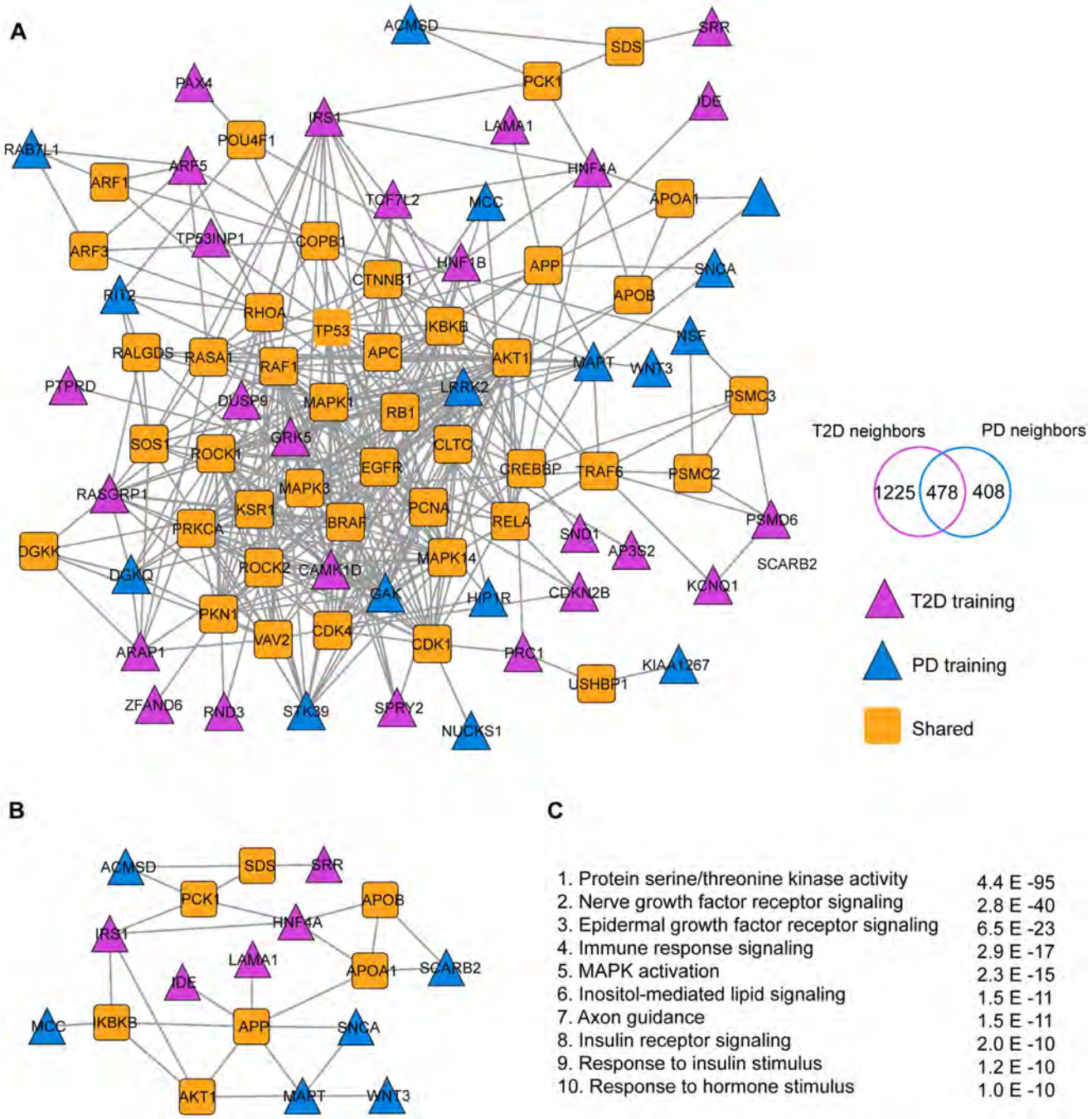

Figure 2. Functional linkage network for Parkinson's disease and type 2 diabetes. A. Network visualization of the top 200 shared genes (orange rectangles) closely associated with training genes associated with Parkinson's disease training genes (blue triangles) and type 2 diabetes (purple triangles) within the FLN (displayed in gray). Venn diagram analysis of shared neighboring genes in Parkinson's disease and type 2 diabetes. **B**. Subnetwork visualization of interactions among confirmed Parkinson's disease and type 2 diabetes genes with *APP*. **C**. Overrepresented pathways identified in Parkinson's disease and type 2 diabetes, as retrieved by Genemania. PD = Parkinson's disease, IFG = pre-diabetes and T2D = type 2 diabetes.
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the HBS cohort. Correlation of biomarker expression with medication was not determined since most of the patients with Parkinson's disease were medicated with several drugs and the number of untreated patients was too small to reliably detect a significant change. Receiver operating characteristic (ROC) analysis revealed that app could distinguish Parkinson's disease patients from healthy controls with a diagnostic accuracy of 80% in the HBS cohort (95% confidence interval, 0.65–0.85, AUC = 0.80, $p<0.0001$) and PROBE study (95% confidence interval, 0.71–0.88, AUC = 0.81, $p<0.0001$).

Discussion

The ultimate goal of network biology is to integrate genomic and biological data to aid in the understanding of complex

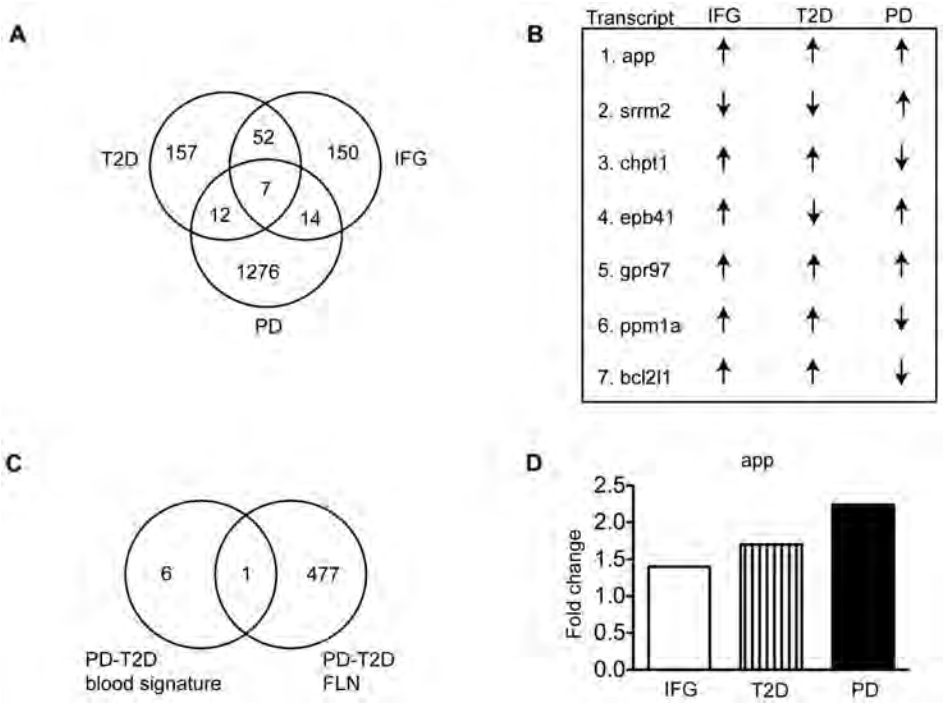

Figure 3. Identification of a blood signature of Parkinson's disease and type 2 diabetes. A. Venn diagram analysis of gene expression data from pre-diabetes, type 2 diabetes and Parkinson's disease patients revealed seven genes common to all groups. **B**. Fold change direction for the seven genes signature identified in the microarrays studies. **C**. Venn diagram analysis of the seven genes dysregulated in blood of Parkinson's disease and type 2 diabetes compared to the Parkinson's disease-type 2 diabetes network. **D**. Fold change in mRNA expression of *APP* in microarray studies. PD = Parkinson's disease, IFG = pre-diabetes and T2D = type 2 diabetes.
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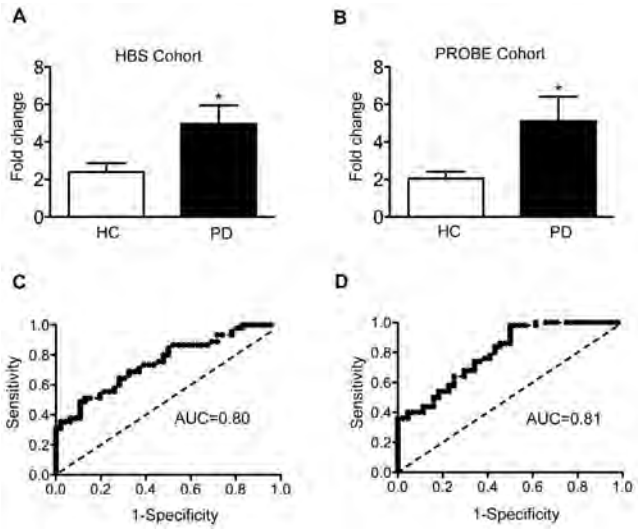

Figure 4. Evaluation of *APP* as a biomarker for Parkinson's disease. A. Quantification of app mRNA in blood of Parkinson's disease patients compared to healthy controls in samples from the HBS cohort. **B**. Replication of app mRNA expression in an independent set of samples from the PROBE study. Fold change was calculated using gapdh as a reference gene and healthy controls as a calibrator. Error bars represent standard error. **C**. ROC curve to evaluate the performance of app as a diagnostic biomarker in the HBS cohort. **D**. ROC curve to evaluate the performance of *APP* expression as a diagnostic biomarker and in the PROBE cohort. (*$p<0.01$). PD = Parkinson's disease, HC = healthy controls and AUC = area under the curve.
doi:10.1371/journal.pone.0083940.g004

diseases. Ideally, integrative network analysis should enable the discovery of reliable biomarkers and ultimately, therapeutic targets for validation. Here we used an integrative network biology approach to better understand the shared molecular networks in Parkinson's disease and type 2 diabetes. The implementation of the RWR within the FLN to prioritize genes allows us to explore the interconnection between both chronic diseases by considering functional associations. Importantly, the RWR algorithm provides a better performance compared to other network-based algorithms such as the direct neighborhood, graph summarization, Markov clustering and network flow [16,26].

Integration of genetic networks revealed a molecular cluster comprising 478 genes closely associated with confirmed Parkinson's disease and type 2 diabetes genes. These findings suggest that genes associated with type 2 diabetes can be used to identify genes associated with Parkinson's disease and vice versa. Biological and functional analysis identified the protein serine-threonine kinase activity, nerve growth factor receptor signaling, activation of the immune response, MAPK cascade, lipid signaling, insulin receptor signaling and response to insulin stimulus, as convergent pathways.

Impaired insulin signaling, glucose intolerance and diabetes have been associated with the development and worsening of motor symptoms in Parkinson's disease [27]. Altered expression of genes and metabolites in blood are expected to reflect a systemic response to the impairment of these processes and thereby providing sensitive indicators of disease pathology. In support of this idea, peripheral blood microRNAs are predictive and reflective of metabolic health and disease in type 2 diabetes [25]. Likewise, transcriptional profiling studies from whole blood have identified several molecular signatures associated with Parkinson's disease [21,22,28,29].

Based on these findings, we interrogated several microrray studies from pre-diabetes, type 2 diabetes and Parkinson's disease patients to investigate whether similar changes in gene expression in whole blood exist between both diseases. Integration of these studies revealed a panel of seven genes significantly dysregulated in blood of patients with pre-diabetes, type 2 diabetes, and Parkinson's disease. Among this group, is the serine/arginine repetitive matrix 2 (*SRRM2*), a splicing factor with altered expression in blood and the substantia nigra of Parkinson's disease patients [29]. In the context of aberrant splicing, a subset of splice variants have been associated with Parkinson's disease in samples from two independent clinical trials, thus suggesting a key role of alternative splicing in Parkinson's disease [21,30].

Another gene with altered expression in blood of pre-diabetes, type 2 diabetes and Parkinson's disease patients is *APP*. Interestingly, the expression of app mRNA in blood is significantly upregulated in pre-diabetes [25] and Parkinson's disease patients [21]. These results suggest that elevated levels of *APP* in blood of type 2 diabetes may be an indicator of neurodegeneration. Therefore, expression of *APP* in blood may be useful to identify type 2 diabetes patients at risk to develop Parkinson's disease.

In order to confirm these findings, we evaluated *APP* expression in blood of patients with Parkinson's disease from two independent cohorts of study participants. Consistent with the microarray data, gene expression levels of *APP* were upregulated in blood of Parkinson's disease patients compared to healthy individuals. Dysregulation of *APP* in blood of Parkinson's disease patients is interesting given its involvement in several neurological disorders. For example, mutations in *APP* linked to familial Alzheimer's disease increase the extracellular concentration of amyloid β protein (Aβ) *in vivo* [31]. More recently, cerebrospinal fluid (CSF) concentrations of Aβ peptides have been widely used to study Alzheimer's disease pathology *in vivo* and their utility to diagnose Parkinson's disease with dementia is under evaluation [32]. In addition to Alzheimer's disease, other neurological disorders including Down's syndrome, autism, and epilepsy are characterized by elevated expression of *APP* [33].

The mechanism by which *APP* increases susceptibility to Parkinson's disease in patients remains unknown. One study found that Aβ peptides enhanced the aggregation of α-synuclein and exacerbated neuronal and motor deficits in a transgenic mouse model [34]. Accordingly, expression levels of Aβ peptides in CSF are associated with motor deficits in early stage Parkinson's disease [35]. Thus, altered processing of Aβ peptides may contribute to neurodegeneration in PD. In a network-based study similar to this, *APP* was identified as a negative regulator of insulin abundance in plasma of mice and a potential link between Alzheimer's disease and type 2 diabetes was suggested [36]. This finding is interesting in light of the recent studies that suggest the involvement of insulin resistance and diabetes in Parkinson's disease [1,23,37]. A potential link between APP processing, insulin regulation and neurodegeneration warrants further investigation.

There are several caveats that should be kept in mind when interpreting the results of this study. Although validation of *APP* in two independent cohorts of patients is a major advance in our study, unanticipated confounds may bias the results. For example, differences in blood counts and Parkinson's disease medications may bias gene expression results. Evaluation of *APP* expression in *de novo* Parkinson's disease patients and in a large well-characterized prospective study will be important to determine the validity of these results. Importantly, given that metabolic impairment plays an early role in the development of Parkinson's disease [38], determining whether *APP* expression is useful for distinguishing individuals at risk for Parkinson's disease, for progression of Parkinson's disease and/or for distinguishing sub-categories of Parkinson's disease patients will be important for future research.

Collectively, the findings provided in this study raises important biological questions. First, the knowledge of many disease comorbidities is limited and is primarily supported by epidemiological studies. In this regard, a potential link between Parkinson's disease and type 2 diabetes has been challenged by several epidemiological studies [39,40] and the evidence of this association is not conclusive [41]. We overcome this challenge by demonstrating that Parkinson's disease and type 2 diabetes are highly interconnected at the molecular level. Importantly, given the involvement of *APP* in insulin regulation and neurodegeneration, its upregulation in blood of Parkinson's disease and type 2 diabetes provides a novel link between both diseases. Evaluation of *APP* as a potential predictor of neurodegeneration in type 2 diabetes is warranted. We foresee this study will provide a platform to generate novel hypothesis and therapeutic strategies for both devastating diseases. With the increasing amount of data deposited in disease databases, network biology provides a cost-effective tool for the discovery of biomarkers and therapeutic targets for validation.

Supporting Information

Table S1 Genes identified by GWAS associated with Parkinson's disease. Genes with a genome-wide significance level of $p<10^{-08}$ were included in this study.
(DOC)

Table S2 Genes identified in GWAS associated with type 2 diabetes. Genes with a genome-wide significance level of $p<10^{-08}$ were included in this study.
(DOC)

Table S3 RWR scores for 200 top-ranked genes according to GPEC.
(DOC)

Table S4 Microarray data for the transcripts dysregulated in pre-diabetes, type 2 diabetes and Parkinson's disease. FC is the log 2-fold change.
(DOC)

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Author Contributions

Conceived and designed the experiments: JAS JAP. Performed the experiments: JAS. Analyzed the data: JAS JAP. Wrote the paper: JAS JAP.

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The emerging role of nutrition in Parkinson's disease

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in ageing individuals. It is now clear that genetic susceptibility and environmental factors play a role in disease etiology and progression. Because environmental factors are involved with the majority of the cases of PD, it is important to understand the role nutrition plays in both neuroprotection and neurodegeneration. Recent epidemiological studies have revealed the promise of some nutrients in reducing the risk of PD. In contrast, other nutrients may be involved with the etiology of neurodegeneration or exacerbate disease progression. This review summarizes the studies that have addressed these issues and describes in detail the nutrients and their putative mechanisms of action in PD.

Keywords: Parkinson's disease, nutrition, neurodegeneration, neuroprotection, antioxidants

INTRODUCTION

Parkinson's Disease is a neurodegenerative disease that usually develops late in life and is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Most cases of Parkinson's disease (PD) are idiopathic since their cause is unknown. Genetic susceptibility and environmental factors (Warner and Schapira, 2003) that mediate mitochondrial dysfunction, inflammation, abrogation of the autosomal-lysomal autophagy system (Beal, 2003), and endoplasmic reticulum stress (Ryu et al., 2002) play a role in disease development.

A growing body of evidence suggests that nutrition may play an important role in PD. Epidemiological and biochemical studies have recently identified promising components in certain food groups that may elicit neuroprotection in PD (Searles Nielsen et al., 2013; Shaltiel-Karyo et al., 2013). However, inclusion or exclusion of other food groups may trigger or exacerbate neurodegeneration. In this review, we focus on the role nutrition plays in promoting or slowing PD.

NUTRIENTS THAT MAY BE ASSOCIATED WITH AN INCREASED RISK OR PROGRESSION OF PD

DAIRY PRODUCTS

Dairy product consumption and drinking milk may increase one's risk of PD independently of calcium intake (Hellenbrand et al., 1996b; Chen et al., 2002; Park et al., 2005; Kyrozis et al., 2013), particularly in men (Chen et al., 2007a). Nonetheless, a positive association between milk consumption and PD risk was also observed in women in one study (Saaksjarvi et al., 2013). Preliminary research shows that individuals who consume large amounts of dairy products may often have low serum uric acid levels (Choi et al., 2005a). Serum urate and uric acid is inversely correlated with the risk of PD and disease duration (Weisskopf et al., 2007; Schlesinger and Schlesinger, 2008; Andreadou et al., 2009; Shen et al., 2013). The neuroprotective effects of serum urate may be limited to men (Gao et al., 2008; Shen et al., 2013) since the same is not observed in women (O'Reilly et al., 2010). In addition, the possible presence of dopaminergic neurotoxins, including pesticides and polychlorinated biphenyls in dairy products may increase the risk of PD (Chen et al., 2002). Accordingly, postmortem studies show higher levels of organochlorines, including dieldrin, an organochlorine pesticide, and polychlorinated biphenyls in the brains of PD patients compared to non-neurological controls (Fleming et al., 1994; Corrigan et al., 1998). Yet, the presence of dopaminergic neurotoxins may not be the only component responsible for the relationship between dairy products and PD. In fact, a strong positive association with the consumption of milk, but not cheese or yoghurt has been reported (Kyrozis et al., 2013). Therefore, other constituents in milk may be detrimental with regards to PD and additional studies are needed in order to identify them. The association between dairy products and PD should be interpreted with caution, however, as other studies have found conflicting results (Miyake et al., 2011c).

NUTRIENTS THAT MAY BE ASSOCIATED WITH A DECREASED RISK OR PROGRESSION OF PD

PHYTOCHEMICALS

The health benefits associated with the intake of phytochemicals present in fruits and vegetables leads to decreased functional decline associated with aging and may slow the progression of PD (Liu, 2003). Epidemiological studies found that high intake of fruits, vegetables and fish was inversely associated with PD risk (Gao et al., 2007; Okubo et al., 2012). Dietary patterns,

characteristic of a Mediterranean diet, are emerging as a potential neuroprotective alternative for PD (Alcalay et al., 2012).

Most fruits and vegetables are rich sources of antioxidants, including vitamins A, B (riboflavin), C, and E, which are present in low levels in some PD patients. Numerous studies have reported a decrease in peroxidase (Ambani et al., 1975), glutathion-peroxidase activities (Kish et al., 1985), and glutathione (Riederer et al., 1989) in the SN of PD patients post-mortem; suggesting metabolic failure in antioxidant mechanisms and chemical processes can lead to lipid peroxidation and parkinsonian characteristics (Uttara et al., 2009).

Although the antioxidant capacity of some fruits and vegetables is evidenced in numerous studies, a recent investigation raised caution about the antioxidant properties of pomegranate. Contrary to the previously reported neuroprotective effects observed in Alzheimer's Disease (Hartman et al., 2006), pomegranate juice exacerbated oxidative stress and neurodegeneration in a rotenone model of PD (Tapias et al., 2013). However, the authors suggest that oxidative stress in a rotenone model may be substantially overwhelming and promegranate may act as a pro-oxidant.

Epidemiological studies have found a decrease in PD risk in individuals who consume foods containing carotenoids and β-carotene (Miyake et al., 2011a). Carotenoids possess antioxidant properties; they act as a reducing agent by protecting lipids through oxidation interference and free radical entrapment (Paiva and Russell, 1999). In mice, pretreatment with β-carotene partially protected against MPTP-induced neurotoxicity (Perry et al., 1985; Yong et al., 1986), but not in primates (Perry et al., 1987). Lycopene, another carotenoid compound, reduces oxidative stress and cognitive decline in a rotenone-induced rodent model of PD (Kaur et al., 2011). One should be cautious however about applying conclusions from animal models about the benefits of carotenoids to humans, since most animals do not absorb or metabolize carotenoids in a similar manner (Paiva and Russell, 1999).

Riboflavin is an integral component of the coenzymes flavin adenine dinucleotide and flavin mononucleotide. Flavin coenzymes participate in oxidation–reduction reactions where they are a major source of energy and are critical for carbohydrate, fat and protein metabolism (Massey, 2000). It has been suggested that riboflavin may be involved in glutathione depletion, cumulative mitochondrial DNA mutations, disturbed mitochondrial protein complexes, and abnormal iron metabolism (Coimbra and Junqueira, 2003). Despite these characteristics, some studies found that riboflavin is not associated with the risk of PD (Abbott et al., 2003; Murakami et al., 2010b), whereas another study observed improved motor skills in PD patients with daily supplementation of riboflavin for 6 months and elimination of red meat (Coimbra and Junqueira, 2003). However, several limitations of this study including omission of a placebo control group and the investigators not being blinded have lead others to question these findings (Ferraz et al., 2004). Another important consideration is that lower protein consumption may affect the absorption of levodopa (Pare et al., 1992; Crevoisier et al., 2003). Therefore, the apparent benefit in motor skills could have resulted from a better absorption of levodopa as opposed to riboflavin supplementation (Ferraz et al., 2004). In addition, intake of other related B vitamins including folate, vitamin B6 and B12 are not associated with a risk of PD (Chen et al., 2004). However, low intake of vitamin B6 is associated with an increased risk of PD (Murakami et al., 2010b). Larger placebo controlled blinded studies done over a longer period of time would be beneficial for determining if riboflavin or other related B vitamins are useful supplements for PD patients.

Recently, dietary intake of nicotine-containing vegetables from edible *Solanaceae* including tomatoes, potatoes, and peppers, was associated with a reduced risk of PD in men and woman who had never smoked cigarettes or tobacco (Searles Nielsen et al., 2013). It remains unclear as to whether the observed protective effect was due to the nicotine content or other components of this group of vegetables. Cruciferous vegetables such as cauliflower, cabbage, and broccoli, are another group of vegetables rich in antioxidants with neuroprotective capacity. For example, sulforaphane and erucin, are potent naturally occurring isothiocyanates found in cruciferous vegetables with antioxidant properties. Treatment with sulforaphane ameloriated motor deficits and protected dopaminergic neurons in a 6-OHDA mouse model of PD (Morroni et al., 2013). Similarly, erucin provided neuroprotective effects by preventing oxidative damage induced by 6-OHDA in an *in vitro* model (Tarozzi et al., 2012). Both, sulforaphane and erucin appear to be promising neuroprotective agents in chronic neurodegenerative diseases (Tarozzi et al., 2013). Taken together, these findings highlight the effects of some vegetables, fruits and constituents they contain as having neuroprotective potential.

Omega-3 (DHA)

Omega-3 polyunsaturated fatty acids (PUFAs) appear to be neuroprotective for several neurodegenerative diseases (Bousquet et al., 2011a). There have been no studies in PD patients that address whether omega-3s are neuroprotective, however, one study showed that supplementation with omega-3 PUFA reduced depression in PD patients (Da Silva et al., 2008). Current research focuses specifically on the omega-3 fatty acid docosahexaenoic acid (DHA). DHA is an essential factor in brain growth and development (Horrocks and Yeo, 1999) and has anti-inflammatory potential due to its ability to inhibit cyclooxygenase-2 (Massaro et al., 2006). DHA protects neurons against cytotoxicity, inhibition of nitrogen oxide (NO) production, and calcium (Ca^{2+}) influx. DHA also increases the activities of antioxidant enzymes glutathione peroxidase and glutathione reductase (Wang et al., 2003). Furthermore, DHA supplementation reduced apoptosis in dopaminergic cells (Ozsoy et al., 2011) and replaced omega-6-PUFAs in the brains of mice post-MPTP treatment (Bousquet et al., 2008). Short-term administration of DHA reduced levodopa-induced dyskinesias in parkinsonian primates by up to 40% (Samadi et al., 2006). Long-term administration of uridine and DHA increased the amount of neural phosphatides in synaptic membranes (Wurtman et al., 2006) and dendritic spines in rodents (Sakamoto et al., 2007). In addition, a reduction in parkinsonian behaviors and elevated dopamine (DA) levels in 6-OHDA rodents was observed after treatment with these supplements (Cansev et al., 2008). Further research on DHA intake in

PD patients is needed to assess whether it is beneficial in slowing disease progression.

The protective effects of DHA are mediated by a metabolic derivative known as neuroprotectin D1 (NPD1) (Bazan, 2009; Serhan and Petasis, 2011). NPD1 protects neurons against oxidative stress, inflammation, the disruption of the cytoskeleton, and from the activation of apoptotic signaling pathways. DHA may protect the brain by increasing glutathione reductase activity that results in decreased accumulation of oxidized proteins (Calon et al., 2004; Wu et al., 2004), lipid peroxide and reactive oxygen species (ROS) (Hashimoto et al., 2005). DHA also inactivates caspase activation signaling pathways (Calon et al., 2005), inhibits hyperphosphorylation of tau (Green et al., 2007) and regulates the PI3K/Akt cascade (Akbar and Kim, 2002). Other potential mechanisms of action of DHA include regulation of inflammation, transcription, and cell membrane properties (De Urquiza et al., 2000; Salem et al., 2000; Jump, 2002).

The precursor to DHA, eicosapentaenoic acid (EPA) is neuroprotective in experimental models of PD (Song et al., 2009; Meng et al., 2010; Taepavarapruk and Song, 2010; Luchtman et al., 2012). In *in vitro* models of PD, EPA attenuated an MPP^+-induced reduction in cell viability and suppressed pro-inflammatory cytokines (Luchtman et al., 2013). A diet rich in EPA diminished hypokinesia induced by MPTP in mice and ameliorated procedural memory deficit (Luchtman et al., 2013).

Because DHA and EPA provide neuroprotection in animal models, more research is warranted to determine if they are beneficial for PD patients. This could be accomplished by following a large group of individuals at risk for PD, some of who are randomly chosen to receive a supplement and other who receive a placebo. The participants could be followed over several years to determine if they develop PD. Alternatively, a large intervention study testing supplements in patients at various stages of PD might reveal whether motor and/or cognitive symptoms are reduced.

Soy (GENISTEIN)

The primary soybean isoflavone genistein is a source of protein that appears to be neuroprotective in ovariectomized rats following 6-OHDA injection, thus suggesting it may be useful for the prevention of PD in post-menopausal women (Kyuhou, 2008). In PD, genistein treatment resulted in dopaminergic neuron protection from lipopolysaccharide (LPS)-induced injury via inhibition of microglia activation (Wang et al., 2005). Genistein pretreatment improved spatial learning and memory in parkinsonian rats (Sarkaki et al., 2009) and restored tyrosine hydroxylase (TH), dopamine transporter (DAT) and Bcl-2 mRNA expression in the midbrain of MPTP-treated animals (Liu et al., 2008). Restored levels of DA and its metabolites, dihydroxyphenylacetic acid, and homovanillic acid, in the striatum were also observed after genistein administration. Additionally, genistein attenuated rotational behavior, protected SNpc neurons (Baluchnejadmojarad et al., 2009), and preserved motor function (Kyuhou, 2008) from 6-OHDA toxicities. Genistein's neuroprotective actions may regulate mitochondria-dependent apoptosis pathways and suppress ROS-induced NF-κB activation (Qian et al., 2012). These studies suggest that it may be worthwhile to test the neuroprotective benefits of genistein in a clinical trial.

CAFFEINE

Caffeine is one of the most widely consumed substances. The health promoting benefits of caffeinated beverages is supported by numerous epidemiological studies (Prakash and Tan, 2011; Tanaka et al., 2011). An inverse association between PD and coffee, and caffeine from non-coffee sources, has been reported (Hellenbrand et al., 1997; Fall et al., 1999; Ascherio et al., 2001). In general, animal studies also indicate that caffeine is neuroprotective. The administration of caffeine to maneb- and paraquat-treated rodents reduced the number of degenerating dopaminergic neurons, microglial cells and nitrite content, while normalizing expression of IL-1β, p38 MAPK, NF-kB, and TK (Kachroo et al., 2010; Yadav et al., 2012). Acute and chronic administration of caffeine also reduced the effect of MPTP (Chen et al., 2001) and 6-OHDA treatment on striatal DA loss (Joghataie et al., 2004) and motor dysfunctions (Joghataie et al., 2004; Aguiar et al., 2006) in rats. Caffeine treatment partially restores DA metabolites in rats following 6-OHDA lesions (Aguiar et al., 2006), and provides neuroprotection in MPTP models of PD (Xu et al., 2010), thus extending its beneficial effects. It is important to note that a caffeine tolerance does not develop with long-term exposure in mice (Xu et al., 2002) and neuroprotection is still apparent with caffeine intake after the onset of neurodegeneration in rats (Sonsalla et al., 2012).

Genetic and pharmacological data from rodent studies indicate that caffeine reduces dopaminergic toxicity and slows disease progression through antagonism of adenosine A_{2A} receptors (Morelli et al., 2010; Prediger, 2010; Xiao et al., 2011; Sonsalla et al., 2012). Inhibition of glutamate neurotransmission using A_{2A} receptor antagonists, may relieve motor symptoms and provide neuroprotection in models of late-stage PD (reviewed in Popoli et al., 2004; Chen et al., 2007b). However, methylxanthine derivatives containing properties of monoamine oxidase B (MAO-B) inhibition, like 8-(3-chlorostyryl) caffeine, may cause oxidative stress via dysfunctional vesicular monoamine transporter 2 (VMAT2) and DA storage mechanisms early in PD (Golembiowska and Dziubina, 2012). Currently, clinical studies are underway to evaluate several A_{2A} receptor antagonists for symptomatic relief and slowing of disease progression (reviewed in Hickey and Stacy, 2011). Caffeine has also shown cytoprotective effects through activation of the PI3K/Akt signaling pathway in SH-SY5Y cells (Nakaso et al., 2008). Therefore, caffeine's ability to down-regulate NO production, neuroinflammation, and microglial activation through these pathways may contribute to neuroprotection (Yadav et al., 2012). It is not fully established, however, that caffeine's neuroprotective role is the sole reason for reduced risk of PD. Nor is it known whether the association is causal rather than reverse causation; the protective effect of caffeine could also reflect an effect of symptoms of PD on caffeine consumption.

Estrogen has significant effects on caffeine's neuroprotective capabilities. Epidemiological studies have consistently demonstrated a greater improvement in male than female Parkinson's patients (Ascherio et al., 2001; Costa et al., 2010).

Interestingly, post-menopausal women who are not taking hormone-replacement therapy receive the same neuroprotective benefits as men (Ascherio et al., 2001). However, high caffeine consumption was associated with an increased risk of PD among women using hormones (Ascherio et al., 2003). More recently, findings from a larger prospective study are consistent with a neuroprotective effect of caffeine intake in men and an attenuated effect in women due to hormone replacement therapy (Palacios et al., 2012a). With regards to animal models, estrogen and caffeine co-administration in MPTP-treated mice, prevented neuroprotection in males and females (Xu et al., 2006). Together these studies suggest that the beneficial effects of caffeine may be limited to men and post-menopausal women not receiving hormone-replacement therapy. However, an open-label study examining caffeine's symptomatic effects and tolerability in patients demonstrated improved non-motor aspects of PD with no gender differences (Altman et al., 2011). Currently adenosine A_{2A} antagonists and caffeine are in phase II and III clinical trials for the symptomatic treatment of PD (Barkhoudarian and Schwarzschild, 2011).

TEA

Several epidemiological studies have addressed the influence of drinking tea (Camellia sinensis) on the risk of PD. A case-control study of Chinese PD patients showed that regular tea drinking protects against PD (Chan et al., 1998). Another study complimented the Chinese PD study showing a reduced risk for PD with tea consumption (two cups/day) (Checkoway et al., 2002). Similarly, a large prospective study showed a reduced risk of incident PD in subjects who habitually drank three or more cups of tea per day (Hu et al., 2007). A retrospective study associated drinking of more than three cups of tea per day with a delayed onset of motor symptoms in Israeli PD patients (Kandinov et al., 2009). Unfortunately, no distinction between green and black tea was made in these studies.

Several reports have revealed that both black and green tea exert neuroprotective effects in PD animal models (Bastianetto et al., 2006; Chaturvedi et al., 2006). Polyphenols in green and black tea extracts provide highly potent antioxidant-radical scavenging activities in brain mitochondrial membrane fractions (Zhao, 2009). In addition, polyphenols in tea reduce occurrence of disease and provide neuroprotection in cell culture and animal models (Nie et al., 2002; Pan et al., 2003b; Guo et al., 2007). In black tea, the polyphenol theaflavin (TF) possess a wide variety of pharmacological properties including antioxidative, antiapoptotic, and anti-inflammatory effects (Aneja et al., 2004; Gosslau et al., 2011). TF-mediated neuroprotection against MPTP-induced dopaminergic neurodegeneneration in rodents was evidenced by increased expression of nigral TH, DAT and reduced expression of apoptotic markers (Anandhan et al., 2012).

Similarly, the polyphenol (–)-epigallocatechin-3-gallate (EGCG) in green tea shows promise in neuroprotection, but one study showed that green tea drinking was unrelated to the risk of PD (Tan et al., 2008). EGCG inhibits nitric oxide and tumor necrosis factor-α secretion from LPS-activated microglia in dopaminergic mesencephalic cells (Li et al., 2004). Given that microglia play a key role in the generation of free radicals and inflammatory factors in the brain, EGCG was classified as neuroprotective *in vivo* (Li et al., 2004). Additionally, EGCG improved cell viability and attenuated MPP-induced intracellular ROS formation via the SIRT1/PGC-1α signaling pathway in MPP induced PC12 cells (Ye et al., 2012). EGCG reduced neuronal cell death and induced nitric oxide synthase (NOS) expression in an MPTP mouse model of PD, thus providing further evidence for its neuroprotection via NO reduction (Kim et al., 2010). Oral pretreatment with EGCG prevented dopaminergic neuron loss in MPTP-treated mice (Levites et al., 2001). In contrast, another study found subtle symptomatic relief but no neuroprotection with similar dose of EGCG in rats following a 6-OHDA lesion (Leaver et al., 2009). The differences between the results from these two studies may reflect the different mechanisms by which MPTP and 6-OHDA exert their neurotoxic effects. Also, the poor bioavailability of oral EGCG in rats may explain why similar doses led to different results in animal models (Kim et al., 2000).

Computational molecular modeling has shown that EGCG is a potent, non-competitive inhibitor that invokes various cellular neuroprotection/neurorescue mechanisms (Zhu et al., 2008). EGCG's mechanisms of action include iron-chelation, scavenging of oxygen and nitrogen radical species, activation of protein kinase C (PKC) signaling pathway and expression of pro-survival genes (Weinreb et al., 2009), and restoration of reduced PKC and extracellular signal-regulated kinases (ERK1/2) activities caused by 6-OHDA toxicity (Zhao, 2009). Tea and/or EGCG prevent neurotoxin-induced cell injury (Weinreb et al., 2004), MPTP-induced dopaminergic neurodegeneration and restore striatal levels of DA and its metabolites (Levites et al., 2001; Choi et al., 2002). Green tea polyphenols could also protect dopaminergic neurons against MPTP-induced injury by exerting inhibitory effects on DA-transporters, which block the uptake the metabolite MPP+ (Pan et al., 2003a).

In summary, tea consumption seems to be a promising lifestyle choice that may slow age-related deficits and neurodegenerative diseases. Given the evidence from preclinical studies, green tea polyphenols are currently being tested as a treatment for de novo PD patients (ClinicalTrials.gov identifier: NCT00461942).

ALCOHOL

Alcohol may exert neuroprotective effects in PD. One case-controlled study found an inverse association between total alcohol consumption and PD (Ragonese et al., 2003). A recent study suggests that low to moderate beer consumption may be associated with a lower PD risk, whereas greater liquor consumption may increase the risk of PD (Liu et al., 2013). Contrary to these findings, most of the epidemiological studies do not support an association between alcohol consumption and risk of PD (Benedetti et al., 2000; Checkoway et al., 2002; Hernan et al., 2003; Palacios et al., 2012b). Currently, the association between alcohol consumption and the risk of PD remains poorly understood.

Despite the conflicting results from epidemiological studies, specific components found in red wine including resveratrol and quercetin, may elicit neuroprotection against PD. Administration of resveratrol or quercetin before MPTP treatment reduced apoptotic cell death and modulated expression of Bax and Bcl-2 in PC12 cultures (Bournival et al., 2009, 2012). Resveratrol

has elicited neuroprotective effects by preventing behavioral, biochemical, and histopathological changes that occur in PD animal models (Bureau et al., 2008; Khan et al., 2010). A diet containing resveratrol protects dopaminergic neurons and attenuates motor coordination in MPTP rodent models (Blanchet et al., 2008; Lu et al., 2008). Many studies suggest that the antioxidant actions of resveratrol are responsible for the neuroprotection from MPP+ toxicity (Alvira et al., 2007; Okawara et al., 2007).

Mechanistically, resveratrol reduces inflammation by trapping free radicals and preventing apoptosis of DA-producing neurons (Blanchet et al., 2008; Jin et al., 2008; Lu et al., 2008). *In vitro* studies showed that resveratrol protects DA neurons against LPS-induced neurotoxicity through the inhibition of microglial activation and subsequent pro-inflammatory factors (Zhang et al., 2010). Resveratrol-mediated neuroprotection has also been attributed to the inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and possibly activation of SIRT1 (Pallas et al., 2009; Zhang et al., 2010). However, one study suggested that SIRT1 activation does not play a major role in the protective effect of resveratrol against MPP+ cytotoxicity (Alvira et al., 2007). Although the evidence from *in vitro* and animal studies is promising, epidemiological studies do not support an association between red wine consumption and PD (Palacios et al., 2012b). Further research on the type and amount of dietary alcohol intake and the risk of PD would be very beneficial.

NUTRIENTS WITH A QUESTIONABLE ROLE IN PD

FAT

Dietary fat has shown inconsistent results in relation to PD. Rodent studies show diets high in fat exacerbate the progression of parkinsonism by exhibiting increased DA depletion in the SN, striatum, and nigrostriatal pathway (Choi et al., 2005b; Morris et al., 2010; Bousquet et al., 2011b). With regards to humans, epidemiological studies found a higher risk of PD among individuals with greater intake of total animal fat (Logroscino et al., 1996; Anderson et al., 1999; Johnson et al., 1999; Chen et al., 2003), whereas other studies show no significant relationship between PD and animal fat (Hellenbrand et al., 1996a; Chen et al., 2002, 2003; Powers et al., 2003). Moreover, the positive association between fat and PD risk reported earlier (Anderson et al., 1999) was not replicated in a larger study (Powers et al., 2003). Nonetheless, the conflicting results from these studies may be attributed to the specific type of fat in the diet, saturated or unsaturated, which is not always specified. Nor is the amount of animal protein consumed to supply the fat intake discussed.

In animal studies and clinical trials, a ketogenic diet, which is high in fat, provided symptomatic and beneficial disease-modifying activity in PD (Gasior et al., 2006). In fact in a small clinical trial, five PD patients on a hyperketonemia diet that substituted unsaturated for saturated fats showed improvement on the Unified Parkinson's Disease Rating Scale (Vanitallie et al., 2005). It should also be noted that the patients on the ketogenic diet ate only 8% protein. Low protein diets lead to better levodopa bioavailability (Pincus and Barry, 1987). It is therefore possible that the observed improvement may have been due to better absorption of synthetic dopamine in four of the patients since one patient was not taking anti-parkinson medication (Vanitallie et al., 2005). Because of the limited number of patients, the difficulty in adhering to a hyperketonemia diet, and the lack of healthy controls, the authors were not able to rule out a placebo effect. The promising results from this preliminary study suggest that another clinical trial of the ketogenic diet that includes a larger number of patients is warranted.

Dietary intake of PUFAs and monounsaturated fatty acids (MUFAs) might influence the risk of PD (Abbott et al., 2003; De Lau et al., 2005). It has been reported in other disease models that PUFA's have anti-inflammatory and neuroprotective properties (Blok et al., 1996; Simopoulos, 1999; Youdim et al., 2000; Kim et al., 2001) and MUFAs are thought to reduce oxidative stress (Colette et al., 2003; Moreno and Mitjavila, 2003). Unsaturated fatty acids are important constituents of neuronal cell membranes and the fatty acid composition of cell membranes is affected by diet. It has been demonstrated in other disease models that infants and young animals with dietary deficiencies in MUFAs and PUFAs have a decrease in brain function (Fernstrom, 1999; Simopoulos, 1999; Youdim et al., 2000; Moreno and Mitjavila, 2003). Moreover, it has been shown that PUFA intake is consistently associated with lower PD risk, and dietary fats modified the association of PD risk with pesticide exposure (Kamel, 2013; Kamel et al., 2013). Notably, PD was inversely associated with the N-3 precursor α-linolenic acid, an essential fatty acid, in a meta-analysis comprising nine studies (Kamel et al., 2013). The health benefit effects of α-linolenic acid may be due to its potential role in protecting against oxidative stress and inflammation (Hassan et al., 2010; Robinson and Mazurak, 2013; Zhang et al., 2013). These studies suggest that a diet high in PUFAs and low in saturated fats might reduce the risk of PD and protect from the toxic effects of neurotoxins, such as those possibly present in milk.

Alternatively, saturated fat could modify the risk of PD by affecting PUFA metabolism and inducing adverse changes in cell membrane lipid composition (Peers, 1997). Thus, fatty acids may contribute to an increased risk of PD via oxidative stress. PUFAs are concentrated in neuronal membranes and play a role in oxidative radical formation. Lipid peroxidation results in oxidative damage and can modify lipid composition of membranes, potentially leading to neuronal death (Farooqui and Horrocks, 1998). In addition, adverse essential fatty acid composition in the mitochondrial membrane may also induce phosphorylation uncoupling, causing energy failure (Peers, 1997). Thus, a high concentration of PUFAs may contribute to neural oxidative stress through lipid peroxidation. Additionally, PD patients have higher concentrations of PUFA peroxidation metabolites and lower concentrations of PUFA and glutathione in the SN compared to healthy controls, further supporting the hypothesis that energy failure may facilitate the onset and/or progression of PD (Chen et al., 2003). However, higher concentrations of PUFA peroxidation metabolites and lower PUFA may arise from several environmental factors in addition to nutrients.

The importance of fats in the pathogenesis of PD in some patients is suggested by genetic studies. Mutations in *PARK2*, which encodes the PD related factor Parkin, lead to an early onset familial form of PD (Kitada et al., 1998). Parkin is part of the E3 ubiquitin ligase complex that targets specific substrates

for degradation via the ubiquitin−proteasome pathway (Shimura et al., 2000). Recently, it was shown that Parkin is a lipid-dependent regulator of fat uptake in mice and patient cells carrying mutations in *PARK2* (Kim et al., 2011). These studies suggest that genetic mutations in the uptake or breakdown of fat may be associated with PD.

Lipid and cholesterol metabolism may also play a role in the pathogenesis of idiopathic PD, however the association between cholesterol and PD is highly debated (Hu, 2010). Lower plasma cholesterol concentrations (Lamperti, 1991) and decreased cholesterol biosynthesis is observed in cell lines from PD patients (Musanti et al., 1993), suggesting that low levels of cholesterol may play a role in PD development and/or progression. In contrast, higher total serum cholesterol may be associated with a modest slower progression of PD (Huang et al., 2011) and lower iron content in SN and globus pallidus in PD patients (Du et al., 2012). Interestingly, the association with increased cholesterol levels and decreased PD was seen primarily in women (De Lau et al., 2006). One possible explanation about the lack of an association between cholesterol levels and PD in men may be due to the gender differences of plasma concentration levels of the antioxidant coenzyme Q10 (De Lau et al., 2006), which are significantly higher in men than in women (Kaikkonen et al., 1999). In this regard, it should be noted that coenzyme Q10 has shown neuroprotective properties in numerous PD studies (Shults et al., 2004; Cleren et al., 2008). More recently, the total; HDL cholesterol ratio was found to be inversely associated with disease duration, thereby suggesting an effect of cardiometabolic protection in PD (Cassani et al., 2013). The results from this study must be interpreted with caution since no healthy controls were included in the analysis.

The studies cited above reflect our incomplete understanding regarding the association between fat intake and PD. The role that fat plays in PD is most likely related to the type of fat in the patient's diet (De Lau et al., 2005), the patient's HDL/LDL ratio, total cholesterol levels and genetic factors. Ideally, large prospective randomized controlled studies are needed to clarify the associations between fat intake and PD.

MEAT

Meat is another source of animal fat and its consumption may be associated with the incidence of PD (Anderson et al., 1999) but the evidence from prospective studies is limited (Gaenslen et al., 2008). Interestingly, intake of processed meat and sausages was inversely associated with PD risk in women (Saaksjarvi et al., 2013). This finding is surprising given the higher incidence of mortality, cardiovascular diseases, and diabetes associated with processed meat consumption (Micha et al., 2010; Rohrmann et al., 2013). In the case of red meat, a positive association between red meat consumption and PD may be explained by the heme content that may act as a toxin when not digested properly. Heme is found in other meats also but not to the same extent. Hemin increases intracellular iron concentrations and hydroxyl radical production, contributing to iron deposits and mitochondrial damage (Schipper, 2000). In this context, iron intake from dietary nutrients may be related to risk for PD (Powers et al., 2003) but the evidence for this association is conflictive (Logroscino et al., 1998, 2008). Despite the inconsistent results, higher intake of iron is associated with neuroprotection in PD (Miyake et al., 2011b). Notwithstanding the positive results, the authors of this study noted that evaluation of dietary intake for 1 month prior to completing the questionnaire by the participants might not properly represent their typical diets.

CARBOHYDRATES

It has been suggested that carbohydrates increase DA production in the brain by allowing easier passage of the DA precursor, tyrosine, through the blood-brain barrier into cerebrospinal fluid (Fernstrom et al., 1979; Wurtman et al., 2003). Carbohydrates with high glycemic index decrease the risk of PD by an insulin-induced increase in brain DA (Murakami et al., 2010a). A balanced diet of carbohydrate and protein mixture improved motor performance in PD patients (Berry et al., 1991). Yet, epidemiological studies about carbohydrate consumption and PD remain inconclusive. For example, the Nurses Health Study and Health Professionals Follow-up Study reported a non-significant direct association in women and inverse association in men for carbohydrate consumption and PD risk (Chen et al., 2003). In contrast, other studies have shown a positive association for total carbohydrate consumption and PD (Hellenbrand et al., 1996a; Abbott et al., 2003).

High carbohydrate diets are associated with an increased risk of type 2 diabetes (T2DM) (Salmeron et al., 1997a,b; Oba et al., 2013). Interestingly, numerous epidemiological studies indicate T2DM is associated with an increased risk of PD (Schernhammer et al., 2011; Xu et al., 2011; Sun et al., 2012; Cereda et al., 2013) but the evidence presented is conflictive (Simon et al., 2007; Palacios et al., 2011; Noyce et al., 2012). Nonetheless, T2DM is associated with more severe motor symptoms in PD (Kotagal et al., 2013). One possible explanation for the link between both chronic diseases is that alterations in common biological pathways may lead to neurodegeneration in patients with T2DM (Santiago and Potashkin, 2013b). In this regard, emerging research is beginning to elucidate the molecular networks and potential mechanisms implicated in both diseases (Santiago and Potashkin, 2013a; Mattson, 2014; Wang et al., 2014). Since carbohydrates are an important part of people's diets and its high consumption may increase risk for T2DM (Salmeron et al., 1997a,b; Oba et al., 2013) further research on the amount and type of dietary carbohydrates consumed in relationship to the risk of PD would be very beneficial.

VITAMIN D, C, AND E

Vitamin D deficiency is prevalent in PD patients (Sato et al., 1997); yet, it is unclear if a reduction in Vitamin D is a cause or consequence of PD. Vitamin D plays a role in regulating Ca^{2+} homeostasis (Garcion et al., 2002; Chan et al., 2009) and if disrupted, SNpc dopaminergic neuron loss is accelerated (Gleichmann and Mattson, 2011). This suggests that dietary regulation of vitamin D may be effective in protecting individuals from PD or slowing PD progression. In animal and cell culture models of PD, vitamin D supplementation was found to be beneficial in slowing disease progression (Wang et al., 2001; Smith et al., 2006; Holick, 2007). In human studies, however, high consumption of food containing vitamin D increased the risk of PD

(Anderson et al., 1999). More recently, vitamin D3 supplementation stabilized PD patients' motor symptoms, preventing an increase in the Hoehn and Yahr stage, compared to a placebo-controlled group (Suzuki et al., 2013). It remains unknown if a reduction in vitamin D stemming from nutritional deficiencies causes an increase in PD and/or if an environmental factor such as UV radiation or exposure to sunlight plays a role. Therefore, more research needs to be done in order to link vitamin D supplementation and its effective in protecting individuals from PD or PD progression.

Vitamin C or ascorbate is highly concentrated in the central nervous system and its neuroprotective capabilities show promise in reducing lipid peroxidation levels and increasing catalase activity (Santos et al., 2008). Higher intake of vitamin C correlates with an increase risk of PD (Scheider et al., 1997). In contrast, in a case-controlled study, individuals consuming a diet rich in vitamin C showed a 40% reduction of PD risk (Hellenbrand et al., 1996a). Interestingly, in a pilot study in which high doses of vitamin C and E were given to early stage PD patients, a decrease in disease progression was observed (Fahn, 1992). Despite this progress, other studies have not found a significant association between intake of dietary vitamin C or vitamin C supplements and risk of PD (Zhang et al., 2002; Etminan et al., 2005). Collectively, the association of vitamin C and PD risk remains inconclusive and more studies are needed to clarify this association.

Vitamin E supplementation provides protective effects on DA neurons in the SNpc (Roghani and Behzadi, 2001), reduce DA loss (Lan and Jiang, 1997), and protect against paraquat toxicity (Storch et al., 2000; Osakada et al., 2004) in rodents and *in vitro*. Pretreatment with vitamin E reduces lipid peroxidation levels (Lan and Jiang, 1997), but depletion of striatal DA was not attenuated in animals (Gong et al., 1991; Chi et al., 1992). The potential benefits seen in vitamin E may be linked to its chain-breaking capabilities in biological membranes, preventing induced oxidative damage by trapping reactive oxyradicals. Yet, other studies have shown that vitamin E has no protective effects against DA-induced toxicity in PC12 cells (Offen et al., 1996) and only partial protection in MPTP-treated marmosets (Perry et al., 1987). A meta-analysis showed a protective effect against PD in humans with both moderate and high intake of vitamin E (Etminan et al., 2005), with a more significant effect observed in men than women (Zhang et al., 2002). In contrast, clinical trials show no neuroprotective benefits from vitamin E in PD patients (Fernandez-Calle et al., 1992; Lewitt, 1994).

Although researchers have started investigating the effect of individual nutrients through supplements the results of these studies remain inconclusive. Antioxidants are much more effective in combinations and therefore a combination of vitamins may be beneficial, perhaps acting synergistically. Thus, we suggest that choosing a diet that contains a variety of foods that are rich in multiple phytochemicals and other bioconstituents may provide a means of disease management. The total elimination of any one food group is not recommended. Additional prospective nutritional studies should help to resolve this issue.

NUTRITION, THE GENOME, AND THE EPIGENOME

A poor diet will have a negative impact on an individual's health. With regards to neurodegeneration, nutrition affects multiple aspects of neurodevelopment, neurogenesis and the functions of neurons and neural networks (Dauncey and Bicknell, 1999). Nutrition-gene interactions play a critical role in dysfunction and disease (Dauncey, 2012). Individual differences in genes such as single nucleotide polymorphisms, mutations and copy number variants significantly modify the effects of nutrition on gene expression (Dauncey, 2013).

A person's epigenome is just as important as their genome. An individual's epigenome reflects the interaction of the person's genome with their environment. Epigenomic modifications include DNA methylation, which may alter protein-DNA interaction and result in genes being expressed or turned off. Another type of modification is histone modification, which may lead to changes in DNA packaging. Histone modification may also lead to switching a gene on or off by making the DNA packaging more or less accessible to proteins. In addition, epigenetic regulation of gene expression through small non-coding RNAs is environmentally regulated. Epigenetic regulation of gene expression plays an important role in development and pathological processes (Dauncey et al., 2001; Babenko et al., 2012; Dauncey, 2012; Hackett et al., 2012; Park et al., 2012; Qureshi and Mehler, 2013). What a person eats and drinks will impact their epigenome (Dauncey, 1997, 2012; Langie et al., 2012). Currently the details about how individual nutrients affect the epigenome generally remain unknown. This area of nutrition research is still in its infancy. If we want to improve peoples' health it will be important to emphasize this area of research in the future because epigenetic changes also impact future generations since they may be inherited.

CONCLUSIONS

Currently, there is an abundance of preliminary evidence that indicates that some nutrients may increase an individual's risk for PD, while others may be neuroprotective (**Figure 1**, Supplementary Tables 1, 2). These results are not unexpected

FIGURE 1 | Role of nutrients in PD. Epidemiological and biochemical studies suggest that inclusion or exclusion of certain food groups may elicit neuroprotection or neurodegeneration. Foods are shown on a spectrum. Foods shown in the red promote neurodegeneration and foods in green promote neuroprotection. Foods shown in the middle (or yellow) part of the spectrum have conflicting results and need to be studied further to assess if they play a role in neurodegeneration or neuroprotection.

since nutrients affect mitochondrial energy function and provide vital antioxidant functions that ameliorate the free-radical byproducts of oxidative phosphorylation. A poor diet may lead to increased oxidative stress, which could impede the antioxidant defense system. In contrast, a well-balanced diet rich in a variety of foods, including numerous servings of vegetables and fruits (especially those containing nicotine) and moderate amounts of omega-3 fatty acids, tea, caffeine, and wine may provide neuroprotection.

In spite of promising effectiveness of these nutrients in PD, we lack definitive evidence-based answers as a result of limited large prospective randomized controlled studies designed to address these issues. Indeed, there are several limitations in some epidemiological studies assessing dietary factors and PD that merit further attention. For example, the assumption that dietary patterns remain unchanged over time is a major limitation. Information on diet during development would be very helpful and may weaken or strength a result. In addition, patients with PD may experience non-motor symptoms at early stages such as constipation, dysphagia, depression, and hyposmia that may affect dietary choices and therefore may be responsible for the impairment of nutritional status observed in PD (Ponsen et al., 2004; Barichella et al., 2009). These factors may remain undetected and therefore not properly reported. Incorporation of these critical factors into clinical practice and epidemiological studies will greatly improve the reliability of studies assessing the role of nutrients in PD.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fnagi.2014.00036/abstract

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System-based Approaches to Decode the Molecular Links in Parkinson's disease and Diabetes

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Abstract

A growing body of evidence indicates an increased risk for developing Parkinson's disease (PD) among people with type 2 diabetes (T2DM). The relationship between the etiology and development of both chronic diseases is beginning to be uncovered and recent studies show that PD and T2DM share remarkably similar dysregulated pathways. It has been proposed that a cascade of events including mitochondrial dysfunction, impaired insulin signaling, and metabolic inflammation trigger neurodegeneration in T2DM models. Network-based approaches have elucidated a potential molecular framework linking both diseases. Further, transcriptional signatures that modulate the neurodegenerative phenotype in T2DM have been identified. Here we contextualize the current experimental approaches to dissect the mechanisms underlying the association between PD and T2DM and discuss the existing challenges toward the understanding of the coexistence of these devastating ageing diseases.

Introduction

PD and T2DM are a growing public health concern with devastating effects in the elderly population. The International Diabetes Federation has estimated that over 380 million people worldwide are afflicted by diabetes and this number is expected to climb to 590 million by 2035 (www.idf.org). Especially in the elderly population, the increase in T2DM is expected to lead to a concomitant increase in neurodegeneration. In this regard,

a substantial amount of epidemiological studies suggest that T2DM is a risk factor for several neurodegenerative diseases including Alzheimer's disease (AD) (Yang and Song, 2013) and PD in some ethnic groups (Sun et al., 2012; Cereda et al., 2013; Santiago and Potashkin, 2013c). Although the exact mechanisms that explain the coexistence of T2DM and PD remains unknown, several studies have revealed potential mechanisms underlying this association. These efforts are in part motivated by recent findings that show that drugs to treat diabetic patients may elicit therapeutic effects in patients with PD (Aviles-Olmos et al., 2013a). In parallel, animal models and network approaches to study the potential links between PD and T2DM are beginning to emerge with the hope of finding an effective treatment. In addition, the molecular framework linking both diseases has begun to be elucidated and common transcriptional signatures may provide further insight into the shared biological mechanisms in PD and T2DM. In this review, we discuss the current experimental approaches to study the association between PD and T2DM and the potential therapeutic targets these system models have revealed.

Epidemiological and Clinical Studies in PD and T2DM

Accumulating evidence from epidemiological studies suggest T2DM is a risk factor for PD. Although a potential link between PD and T2DM remain controversial (Palacios et al., 2011; Savica et al., 2012), most of the epidemiological studies indicate a high incidence of T2DM among patients with PD (Santiago and Potashkin, 2013c). Patients with T2DM have a 36% increased risk of developing PD (Hu et al., 2007; Xu et al., 2011). Case-control studies indicate that T2DM is associated with an increased risk of PD in some ethnic groups including Danish, Chinese and Taiwanese (Schernhammer et al., 2011; Sun et al., 2012; Wahlqvist et al., 2012). Similarly, a positive association between

PD and T2DM was indicated in large cohort studies and 62% of PD patients with dementia are insulin resistant (Hu et al., 2007; Xu et al., 2011; Bosco et al., 2012). Notwithstanding the evidence supporting the association between PD and T2DM, there remains uncertainty given the studies that have found inverse associations (D'Amelio et al., 2009; Lu et al., 2014) or no association (Palacios et al., 2011; Savica et al., 2012). One possible factor that may explain the conflictive findings among epidemiological studies is that diagnosis of T2DM is sometimes based on self-report. Another important confounding factor is the impact of drugs used to treat patients with PD and T2DM. For example, PD medications such as levodopa, induces hyperglycemia and hyperinsulinaemia (Van Woert and Mueller, 1971). Further, anti-diabetic drugs such as metformin-inclusive sulfonylurea and exenatide may elicit neuroprotection in PD (Wahlqvist et al., 2012; Aviles-Olmos et al., 2013a). Therefore, larger epidemiological studies taking into account these potential confounding factors will be helpful to better understand the association between PD and T2DM.

Interestingly, conditions linked to T2DM appear to be associated with more severe motor symptoms and conditions in PD patients. Not surprisingly, repeated inpatient care and longer duration of hospitalization is observed in PD patients with T2DM (Scheuing et al., 2013). Insulin resistance, a hallmark feature of T2DM, is associated with an increased risk of dementia in PD (Bosco et al., 2012). In addition, T2DM contributes to postural instability and gait difficulty in PD (Kotagal et al., 2013). Given the fact that these symptoms are manifested later in the disease (Hoehn and Yahr, 1967), T2DM is most likely associated with PD progression. Accordingly, patients with T2DM manifest a

higher United Parkinson's Disease Rating Scale (UPDRS) and more severe Hoehn & Yahr staging (Cereda et al., 2012). Collectively, these findings highlight the detrimental impact T2DM imposes on PD patients and raises concerns about the potential implications of T2DM in the clinical management of PD patients. The substantial evidence from epidemiological studies heightens the urgency to better understand the molecular mechanisms underlying this association.

Modeling Complex Disease Comorbidity: PD and T2DM

Modeling disease comorbidities is a challenging task in experimental medicine owing to the multiple factors and interrelated conditions associated with complex diseases. Identifying the triggering factors and mechanisms that lead to the development of concomitant diseases with dissimilar phenotypic features and unknown etiology is very difficult. This is the case of PD and T2DM, both complex multifactorial disorders in which a combination of environmental and genetic factors are involved in disease pathogenesis. Genetic risk factors for PD and T2DM account for approximately 5-10% of the cases. Consequently, a wide range of environmental insults is considered important in the development of both diseases.

Several animal models have been proposed to study idiopathic PD. The most common animal models are designed to produce nigrostriatal dopaminergic lesions with environmental toxins including 6-hydroxydopamine (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), paraquat or rotenone. None of these models exactly

recapitulates the clinical symptoms and pathology of PD observed in humans however (Potashkin et al., 2010; Bezard et al., 2013).

In the context of T2DM, animal models include models of insulin resistance and pancreatic beta cell failure, characteristic features of T2DM. Obesity is a risk factor for T2DM, consequently most of the animal models of T2DM are obese (King, 2012). There are both monogenic and polygenic models of obesity used in T2DM research. Monogenic models of obesity include rodent models with severe obesity ($Lep^{ob/ob}$), Zucker diabetic rats (ZDF) and $Lep^{db/db}$ mice with defective leptin signaling and mutations in the leptin receptor, but these models may not accurately reflect T2DM (King, 2012). In contrast, polygenic models of obesity are considered more accurate than monogenic models as they closely mimic the characteristic features of T2DM. Examples of polygenic models of obesity are KK mice that are mildly obese and manifest severe hyperinsulinemia and OLETF rats with mild obesity and late onset hyperglycemia, reviewed in King (2012). In addition to genetic models, high-fat diets are known to induce obesity and insulin resistance in rodents (Winzell and Ahren, 2004).

The models discussed above are designed to mimic PD and T2DM independently but do not recapitulate disease comorbidity. Indeed, understanding the potential link between PD and T2DM has been hampered by the lack of testable models that closely recapitulates existing comorbidity. To address this issue, several studies have studied metabolic abnormalities in rodent models of PD. For example, a 6-OHDA model of PD was used to determine that a high-fat (HF) diet altered insulin signaling, impaired nigrostriatal

dopamine function and exacerbated neurodegeneration (Morris et al., 2010; Morris et al., 2011a). Although the impairment of insulin signaling is anticipated with a HF diet, the results from this study provide evidence for the increased vulnerability of DA neurons in response to HF-diet induced insulin resistance. In addition, nutrient excess and mitochondrial dysfunction are implicated in the development of neurodegeneration in diabetes (Chowdhury et al., 2011).

Similarly, another study investigated whether central and peripheral insulin signaling was altered in a 6-OHDA middle-aged rat model of PD (Morris et al., 2011b). In this study, impaired insulin signaling, as demonstrated by increased phosphorylation of the insulin receptor substrate 2 (IRS2) and decreased phosphorylation of v-akt murine thymoma viral oncogene homolog 1 (AKT, protein kinase B), was observed in the striatum but not in skeletal muscle. Despite the differences observed in insulin signaling in the brain and the periphery, lesioned animals exhibited alterations in glucose and insulin levels at later time points.

More recently, a mouse model expressing a mutant form of human α-synuclein (A53T) in neurons was used to investigate metabolic and physiologic abnormalities in response to a high calorie diet (HCD) (Rothman et al., 2013). Strikingly, A53T mutant mice were resistant to HCD-induced obesity and insulin resistance, thus providing evidence for the involvement of α-synuclein in metabolic dysfunction in PD. The authors noted the importance of evaluating whether mutations in other genetic risk factors for PD would display the same phenotype.

Another approach to studying comorbidities is to expose diabetic mice to environmental toxins associated with PD. For example, diabetic mouse models (ob/ob and db/db) treated with MPTP resulted in the accelerated loss of dopaminergic neurons and increased activation of glial cells in the substantia nigra of db/db mice (Wang et al., 2013). Interestingly, neurodegeneration in this model was accompanied by the increased activation of inflammatory molecules including NRLP3, excess production of IL-1ß and upregulation of monomeric and aggregated forms of α-synuclein in both pancreas and midbrain of T2DM mice. Moreover, markers of endoplasmic reticulum (ER) stress CHOP and GPR78 were upregulated in the pancreas, liver and brain of T2DM mice. In addition to α-synuclein, DJ-1, an antioxidant protein encoded by the PD gene *PARK7*, is upregulated in pancreatic islets of mice under hyperglycemic conditions resulting from a high fat diet (Waanders et al., 2009). More recently, DJ-1 deficient mice developed glucose intolerance and reduced pancreatic β-cell mass, thus indicating that DJ-1 plays a role in glucose homeostasis (Jain et al., 2012).

The findings discussed above highlight important factors in the understanding of the underlying mechanism in PD and T2DM. First, the activation of microglia prior to neuronal degeneration, indicates that neuroinflammation is a triggering factor in dopaminergic cell death and thus may be an early indicator of neurodegeneration in PD. In addition, increased production of inflammatory cytokines may exacerbate neurodegeneration in PD. The increased expression of aggregated α-synuclein in the pancreas and the upregulation of ER-stress markers in the pancreas, liver and brain

suggests that insults in peripheral organs may precede the onset of PD. Collectively, the findings presented above support the hypothesis that shared dysregulated pathways lead to PD and T2DM and that systemic changes may reflect those observed in the brain (Santiago and Potashkin, 2013c). A schematic representation of the most important findings obtained from animal models investigating the link between PD and T2DM is presented in Figure 1.

A Network View of T2DM and Neurodegeneration

High-throughput methods have identified numerous genetic variants associated with PD and T2DM. Increasing amounts of genomic data are deposited in disease databases but the information only allows researchers to examine the association of a single genetic variant at a time. Yet, the individual effect of a single gene is usually not reflective of the complex biological pathways that lead to disease. Complex diseases like PD and T2DM may arise from alterations in multiple genes and biological pathways. To address this issue, network biology has emerged as a paradigm shift in the field of medicine (Furlong, 2013).

The construction of the human disease, functional linkage and metabolic disease networks has expanded our understanding of the underlying mechanisms leading to disease comorbidities (Goh et al., 2007; Lee et al., 2008; Linghu et al., 2009). For example, the human metabolic network revealed that diseases with metabolic links displayed greater comorbidity than those with no metabolic links (Lee et al., 2008). Integrative network analysis uncovered shared genetic and functional modules between

AD, PD and type 1 diabetes (Menon and Farina, 2011). Further, network analysis revealed common functional modules between T2DM and spinal muscle atrophy (SMA) (Rende et al., 2013) and Schizophrenia (Liu et al., 2013).

In the same context, integrated network-based approaches unveiled convergent molecular pathways in PD and T2DM. Interactome mapping of a well-characterized group of genes associated with PD and T2DM uncovered a molecular cluster of more than 400 genes linking both diseases (Santiago and Potashkin, 2013a)(Figure 2A). This comprehensive network analysis identified protein serine-threonine kinase activity, MAPK cascade, activation of the immune response, and insulin receptor and lipid signaling as convergent pathways. Moreover, network analysis of PD blood biomarkers identified the hepatocyte nuclear factor (HNF4α) and tumor necrosis factor (TNF) as central regulatory nodes (Potashkin et al., 2012). In this respect, HNF4α is a key metabolic regulator involved in hepatic gluconeogenesis, lipid metabolism (Palanker et al., 2009) and a risk factor for T2DM (Holmkvist et al., 2008; Bonnefond et al., 2010). Interestingly, HNF4α interacts with peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1α), a potential therapeutic target in PD (Zheng et al., 2010; Shin et al., 2011), and it is involved in several aspects of the hepatic fasting response and gluconeogenesis (Rhee et al., 2003). TNF is associated with inflammation and insulin resistance in T2DM (Monroy et al., 2009).

Network based approaches also provides a framework to prioritize key genetic connections associated with PD and T2DM. Among the top-ranked genes within the PD-

T2DM shared cluster were the creb binding protein (*CREBP*), *AKT1* and amyloid beta (A4) precursor protein (*APP*), implicated in insulin regulation and T2DM (Santiago and Potashkin, 2013a). Not surprisingly, this group of genes is involved in neurodegeneration. For example, reduced CREB signaling appears to be an underlying mechanism for neuronal death in PD (Chalovich et al., 2006) and tauopathies (Ljungberg et al., 2012). Neuroprotective effects afforded by leptin and the liver growth factor observed in 6-OHDA models of PD involve the regulation of CREB signaling (Weng et al., 2007; Gonzalo-Gobernado et al., 2013). Genetic variations in *AKT1* are associated with decreased risk of PD (Xiromerisiou et al., 2008) and dysregulation of endogenous AKT signaling may play a role in α-synuclein expression in PD (Kim et al., 2011). Mutations in *APP*, implicated in familial AD (Goate et al., 1991) cause the dysregulation of APP processing leading to the aggregation of amyloid-β plaques, a prominent pathology in AD. In this regard, numerous studies indicate that amyloid-β deposition is also present in PD patients with dementia (Kalaitzakis et al., 2008; Kalaitzakis et al., 2011; Dugger et al., 2012; Petrou et al., 2012).

In the context of diabetes, *CREBBP* deficiency increases insulin sensitivity, glucose tolerance, and protects from body weight gain induced by a high-fat diet (Yamauchi et al., 2002). Likewise, loss of *AKT1* in mice serves as a metabolic regulator by protecting from diet-induced obesity and its associated insulin resistance (Wan et al., 2012). Similar to *CREBBP* and *AKT1*, *APP* regulates insulin secretion in pancreatic islets and affects plasma insulin levels in mice (Tu et al., 2012). In addition, high glucose levels increased levels of APP protein and Aß peptides in cellular models of AD (Yang et al., 2013).

Interestingly, analysis of the PD-T2DM network topology revealed the interaction of *APP* with the insulin-degrading enzyme (*IDE*), an associated risk factor for T2DM (Zeggini et al., 2007), and *SNCA* implicated in PD (Singleton et al., 2013)(Figure 2A). Homozygous deletions of the *IDE* gene in mice resulted in the degradation of Aß peptides and elevated levels of the intracellular signaling domain of APP, thus providing a common mechanism between hyperinsulinemia, T2DM, and AD (Farris et al., 2003). In addition, Aß peptides enhanced the aggregation of SNCA protein in transgenic mice models, thereby suggesting a link between PD and AD (Masliah et al., 2001). Intriguingly, decreased levels of IDE are associated with increased levels of SNCA protein in human T2DM islets (Steneberg et al., 2013) and increased expression of SNCA in pancreatic beta cells of mice impairs glucose stimulated insulin secretion (Steneberg et al., 2013). One possible mechanism is that SNCA interacts with ATP-sensitive potassium channels (K_{ATP}) and regulates insulin secretion (Geng et al., 2011). Taken together, these findings provide evidence that APP, SNCA and IDE all play a role in insulin regulation and their dysregulation may provide a mechanism by which T2DM patients may develop PD or AD.

It has been hypothesized that analysis of highly dysregulated subnetworks may identify candidate biomarkers and potential therapeutic targets. For example, integrative network analysis identified the protein tyrosine phosphatase non-receptor type 1 (*PTPN1*) as a potential diagnostic biomarker for Progressive Supranuclear Palsy (PSP), an atypical parkinsonian disorder that is often misdiagnosed as PD (Santiago and Potashkin, 2013b).

Quantification of *PTPN1* mRNA in whole blood samples from the Prognostic Biomarker Study (PROBE) clinical trial revealed that *PTPN1* may be used as a biomarker to distinguish PD and PSP patients. Interestingly, PTPN1 is associated with insulin regulation, T2DM, and inflammation (Kenner et al., 1996; Zabolotny et al., 2008) therefore, PTPN1 inhibitors may be potential therapeutic targets for T2DM (Patel et al., 2012). Similarly, integration of gene expression data from multiple studies elucidated a transcriptional signature in blood characteristic of PD and T2DM. A set of 7 mRNAs were identified which are common in blood of pre-diabetes, T2DM, and PD patients including *APP*, *BCL2L1*, *CHPT1*, *GPR97*, *EPB41*, *PPM1A*, and *SRRM2*, (Santiago and Potashkin, 2013a)(Figure 2B). In this study, *APP* mRNA was found to be upregulated in blood of PD patients compared to healthy individuals (Santiago and Potashkin, 2013a)(Figure 2C). Given the role of *APP* in insulin regulation (Rende et al., 2013), and the prevalence of insulin resistance in PD patients (Bosco et al., 2012; Santiago and Potashkin, 2013c), the evaluation of *APP* as a predictor of neurodegeneration in T2DM patients warrants further investigation.

Interactive Databases to Study the Linkage Between PD and T2DM

The development of interactive databases has established a framework for the systematic exploration of gene-disease and disease-disease associations. One example is DisGeNET, a comprehensive database of human genetic associations (Bauer-Mehren et al., 2011). Using DisGeNET, the association between PD and T2DM can be explored by integrating multiple sources of information (Figure 3). Another example is the Integrated Complex Traits Networks (iCTNet) interface that contains results from GWAS published studies

and data from the GWAS catalog is also helpful to dissect shared molecular networks in PD and T2DM (Wang et al., 2011). The main differences between these databases are the sources from which the information is retrieved and how they are curated. For example, DisGeNET integrates genetic associations from multiple curated databases and literature text mining whereas iCTNet integrates information from GWAS, protein-protein interactions, expression data and drug targets. Similarly, the Human Experimental/Functional Mapper (HEFalMp) provides an interface to investigate genetic associations and cross-talk between biological pathways in PD and T2DM using functional maps (Huttenhower et al., 2009). These interactive databases are freely available to the research community and some of them can be access through the Cytoscape software environment (Shannon et al., 2003).

System-Based Studies Reveal Potential Therapeutic Targets in PD and T2DM

Mitochondrial dysfunction is implicated in the pathogenesis of PD and T2DM. Consequently, transcription factors involved in mitochondrial biogenesis, cellular bioenergetics, and energy metabolism, are becoming attractive therapeutic targets for PD and T2DM. One of the most studied is PGC-1α, a transcription factor that plays a key role in mitochondrial biogenesis, fatty acid oxidation, insulin resistance and gluconeogenesis (Puigserver et al., 2003; Koo et al., 2004). Decreased levels of PGC-1α have been reported in the SNpc of PD patients (Zheng et al., 2010) and in skeletal muscle of insulin resistant and T2DM patients (Mootha et al., 2003; Richardson et al., 2005). Repression of PGC-1α by the parkin substrate PARIS leads to neurodegeneration in PD

models, thus suggesting PGC-1α as a potential therapeutic target for PD (Shin et al., 2011).

Similar to PGC-1α*,* altered regulation of PTEN induced putative kinase 1 locus (*PINK1)*, previously associated with PD (Valente et al., 2004; Beilina et al., 2005) is associated with physical inactivity and T2DM (Scheele et al., 2007). Specifically, *PINK1* mRNA is downregulated in skeletal muscle of T2DM and its suppression appears to contribute to altered glucose metabolism (Scheele et al., 2007). Given the role of *PINK1* in cellular energetics, mitochondrial function (Clark et al., 2006; Yang et al., 2006) and glucose metabolism, dysregulation of the *PINK1* locus is thought to be involved in the pathogenesis of T2DM and PD (Scheele et al., 2007). Other putative therapeutic targets for PD are the AMP-activated protein kinase (AMPK) and the silent information regulator T1 (SIRT1), both important cellular metabolic regulators involved in mitochondrial function (Hardie, 2007). It has been proposed that nutrient excess leading to hyperglycemia, may cause a downregulation of mitochondrial oxidative capacity through the AMPK-PGC-1α signaling pathway thus suggesting the involvement of these factors in the development of neurodegeneration and other complications in T2DM (Chowdhury et al., 2011; Chowdhury et al., 2013).

Anti-diabetic drugs are emerging as promising therapeutic agents for PD. One potential therapeutic strategy is treating PD patients with the class of T2DM drugs that target the glucagon-like peptide-1 (GLP-1) receptor activity. For example, exenatide, a GLP-1 agonist that restores glucose homeostasis in T2DM patients (Buse et al., 2004; DeFronzo

et al., 2005), have elicited neuroprotective effects in a clinical trial of PD (Aviles-Olmos et al., 2013a). Exenatide treatment was well tolerated and improved motor and cognitive measures in PD patients. Although, the mechanism by which exenatide promotes neuroprotection is unclear, evidence from PD animal models suggest that exenatide inhibits microglial activation and matrix metalloproteinase-3 (MMP3) expression (Kim et al., 2009). Consequently, inhibition of the inflammatory pathways is suggested to stimulate downstream insulin signaling and ultimately result in neuroprotection in PD (Santiago and Potashkin, 2013c).

Conclusions

Evidence is mounting that indicates impaired insulin signaling, ER-stress and inflammation may be the underlying mechanisms by which T2DM patients develop PD. There is considerable evidence that PD and T2DM share common mechanisms at the cellular and molecular level and that insulin resistance causes neurodegeneration (Aviles-Olmos et al., 2013b; Santiago and Potashkin, 2013c). Several lines of research using animal models indicates that nutrient excess leading to aberrant insulin signaling, insulin resistance and inflammation may precede the onset of PD. Aberrant expression of α-synuclein in peripheral organs and its potential implications in insulin regulation warrants further investigation. Based on the observations from animal studies, common dysregulated processes occur in peripheral organs and the brain suggesting that PD may be highly influenced by systemic changes.

Network modeling approaches have been useful to dissect the molecular networks and biological pathways underlying the association between PD and T2DM. In particular, dysregulation of multiple genetic factors including *APP* in blood of pre-diabetes, T2DM and PD patients may provide a diagnostic tool to identify patients with T2DM at risk of developing PD. Future research is needed to determine whether *APP* modulates the neurodegenerative phenotype in T2DM. In addition, given the involvement of SNCA, IDE and APP in insulin regulation, functional studies to understand this connectivity may reveal the underlying molecular mechanism by which PD and T2DM coexist. Further, network analysis of genetic expression data generated from animal models studying the link between PD and T2DM may capture additional genetic factors associated with this comorbidity. Collectively, network approaches have provided a framework to understand the coexistence of PD and T2DM and to identify candidate genes with clinical utility. It is expected that network analysis will facilitate the discovery of novel therapeutic targets and accelerate the understanding of disease mechanisms. To date, drugs to treat diabetic patients appear to be promising therapeutic agents against PD. Despite the promising results observed with exenatide treatment in PD, these findings needs to be replicated in a larger group of patients. In addition, evaluation of exenatide in patients at risk of PD will be important to determine whether this drug can be useful for prevention.

In summary, the evidence presented in this review supports the association between PD and T2DM. From a clinical perspective, the burden that T2DM impose in the worsening of symptoms and acceleration of PD raise cautionary flags in the clinical management of PD. Therefore, better understanding of the molecular links between T2DM and PD is

expected to open new avenues for the discovery of therapeutics and diagnostic biomarkers that will aid in the disease management.

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Figure legends

Figure 1. Animal models to study the association between PD and T2DM. Evidence from diabetic and PD rodent models indicates that exposure to environmental insults results in central and peripheral impairment of insulin signaling. Environmental toxins associated with PD (MPTP, 6-OHDA), nutrient excess, and a high-fat diet results in the accelerated loss of dopaminergic neurons and metabolic inflammation. Aberrant expression of molecules implicated in ER stress, inflammation, and insulin resistance can be observed in the brain and peripheral organs suggesting that PD may be highly influenced by systemic changes. Red arrows indicate upregulation, and blue arrows indicate downregulation.

Figure 2. Integrated network analysis to identify the molecular framework shared between PD and T2DM. A. Mapping of well-characterized genes associated with PD (blue triangles) and T2DM (purple triangles) into the human functional linkage network revealed that both diseases are highly interconnected at the molecular level. B. Microarray analyses revealed a set of seven genes in common in blood of pre-diabetes, T2DM and PD patients (GSE26168, GSE34287). C. *APP* mRNA is upregulated in blood of PD patients compared to healthy individual in samples obtained from a clinical trial. Figure adapted from (Santiago and Potashkin, 2013a).

Figure 3. Shared genetic associations between PD and T2DM identified with DisGeNET.

Disease-gene networks can be accessed through DisGeNET database. Networks can be merged and further analyzed. Shared genetic connections are displayed in yellow circles. The color of the lines indicates the category of data from which the connection was identified.

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Figure 1

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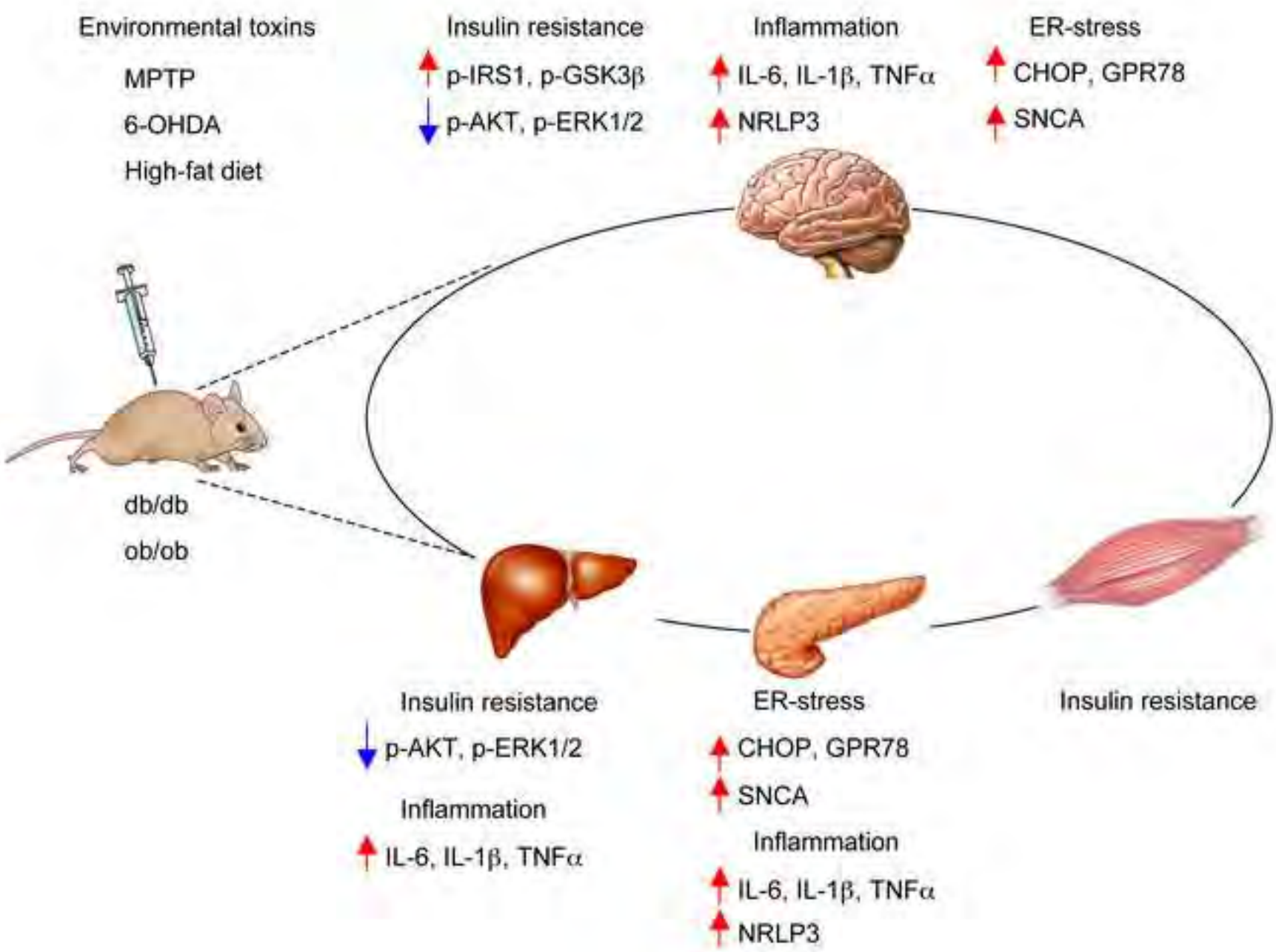

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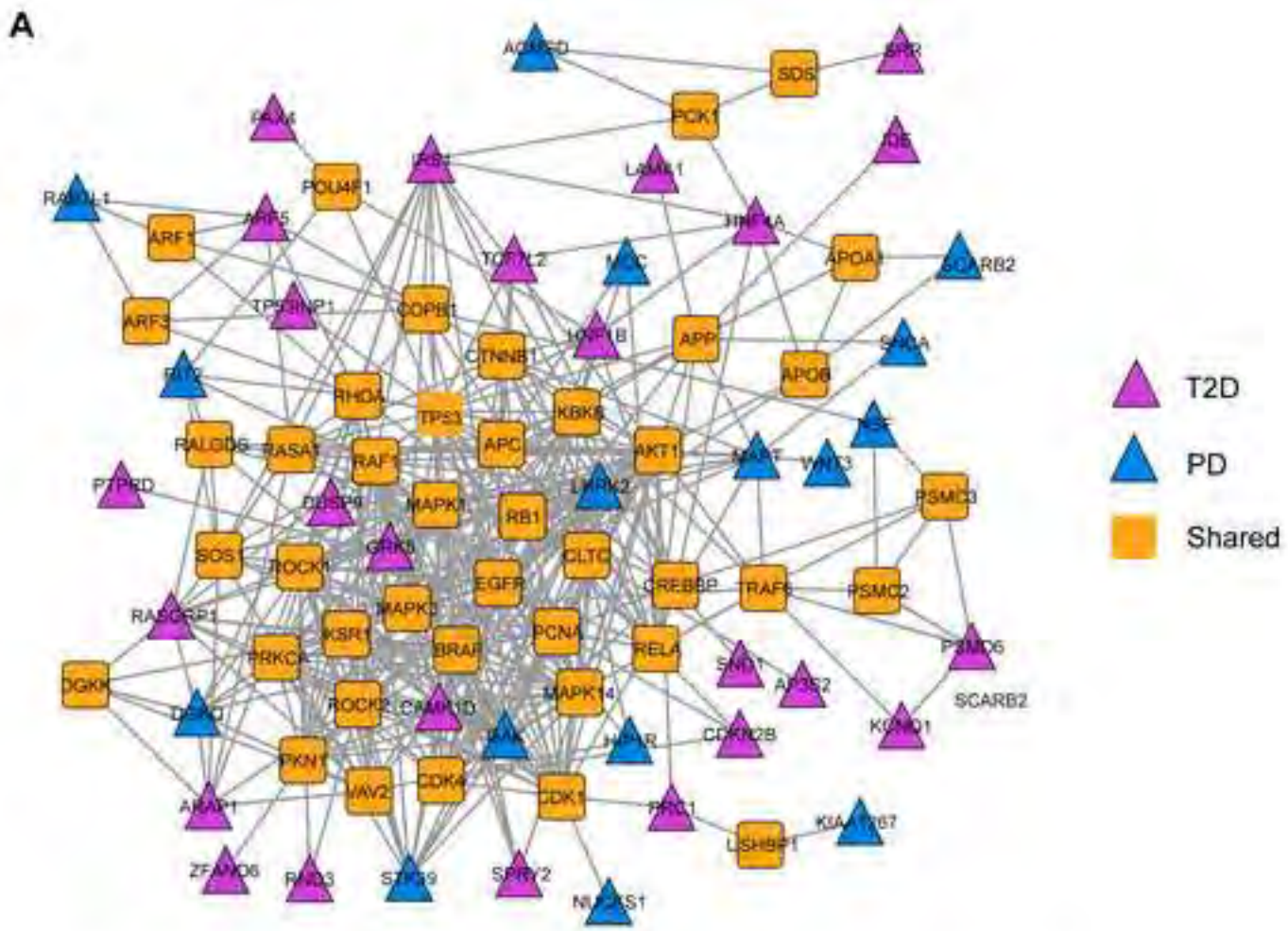

Figure 3

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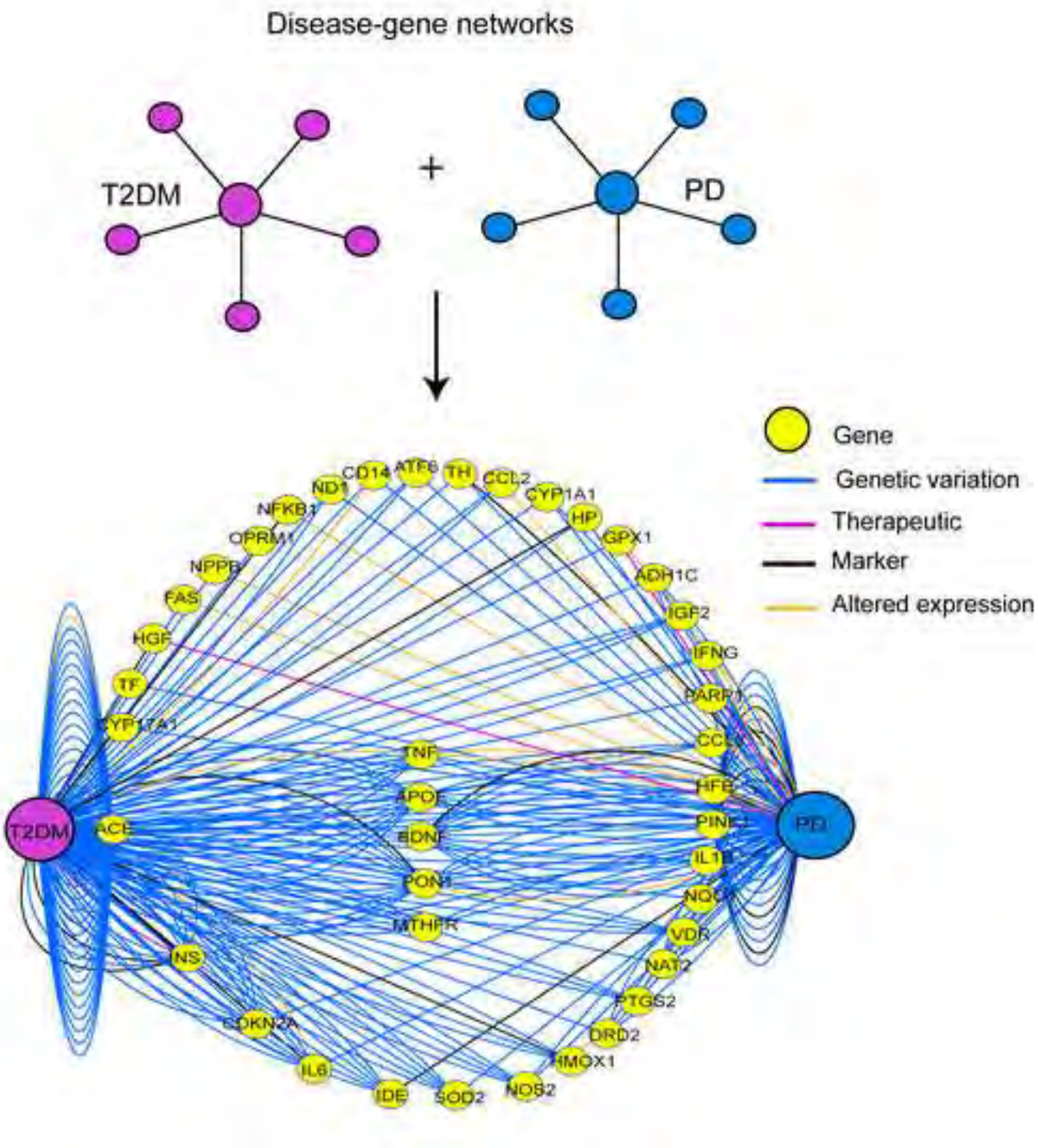

Network analysis identifies *HNF4A* and *SOD2* mRNAs as biomarkers for Parkinson's Disease

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Abstract

Increasing evidence indicates that Parkinson's disease (PD) and type 2 diabetes (T2DM) share dysregulated molecular networks. We identified 85 genetic connections shared between PD and T2DM from curated disease-gene databases. Nitric oxide biosynthesis, lipid and carbohydrate metabolism, insulin secretion and inflammation were identified as common dysregulated pathways. A network prioritization approach was implemented to rank genes according to their distance to seed genes and their involvement in common biological pathways. Quantitative PCR assays revealed that highly ranked genes, *HNF4A* and *SOD2,* are upregulated in PD patients compared to healthy controls in 192 whole blood samples from two independent clinical trials, the Harvard Biomarker Study (HBS) and the Prognostic Biomarker Study (PROBE). Relative abundance of *HNF4A* mRNA significantly correlated with Hoehn and Yahr stage in PD patients, thus revealing its potential as a disease progression marker. This study provides an innovative approach to prioritize biomarkers with clinical utility and biological relevance. Evaluation of these biomarkers in *de novo* PD patients and in a larger prospective longitudinal study is warranted.

Keywords: biomarkers; neurodegeneration; Parkinson's disease; HNF4A; SOD2; Type 2 diabetes; regulatory networks; biological pathways

1. Introduction

Network-based methods have been used to identify previously unrecognized biological pathways, genetic associations, and biomarkers in neurological disorders. For example, integrated network analysis identified genetic nodes associated with late onset Alzheimer's disease (AD)(Zhang, et al., 2013) and biological pathways in multiple sclerosis (Baranzini, et al., 2009). Similarly, a network approach identified *PTPN1* mRNA as a diagnostic biomarker in progressive supranuclear palsy (Santiago and Potashkin, 2013b).

Recently, emerging evidence suggest that perturbations in shared molecular networks may trigger the development of PD and T2DM (Santiago and Potashkin, 2013c). An integrative network-based approach using data from genome-wide association studies (GWAS) was used to investigate the molecular framework linking PD and T2DM and to identify potential biomarkers with clinical applicability. Results from these studies identified the amyloid precursor protein (*APP*) mRNA as a biomarker for PD (Santiago and Potashkin, 2013a).

Here we expanded the network analysis to integrate data from publicly available and curated disease-gene databases to further investigate the connection between PD and T2DM. Shared genetic connections between both diseases were mapped into the human functional linkage network (FLN). We implemented a random walk algorithm with restart (RWR) to establish quantitative associations among the genes shared between PD and T2DM. We further evaluated the applicability of the network prioritization approach

by testing highly ranked genes as diagnostic biomarkers for PD. In this study we identify *HNF4A* and *SOD2* mRNA as biomarkers that can be used to identify patients with PD.

2. Methods

2.1 Database mining and network analysis

We queried the DisGeNET database (Bauer-Mehren, et al., 2011) that integrates information from four respositories: Online Medelian Inheritance in Man (OMIM), UniProt/SwissProte (UNIPROT), Pharmacogenomics Knowledge Base (PHARMGKB), and Comparative Toxicogenomics Database (CTD). DisGeNET can be accessed through the Cytoscape 2.8.3, a platform for complex network analysis (Shannon, et al., 2003). Using the advanced network merge option in Cytoscape, both PD and T2DM gene networks were merged using gene ID as a matching attribute. Genes that are not shared between PD and T2DM were removed from the networks for clarity.

The Disease and Gene Annotations database (DGA) (Peng, et al., 2013) was accessed through the web (http://dga.nubic.northwestern.edu/pages/search.php). We searched for gene annotations to PD and T2DM. Similarly, we explored Human Experimental/Functional Mapper (HEFalMp) using the web-interface (http://hefalmp.princeton.edu/) to investigate genetic associations between PD and T2DM (Huttenhower, et al., 2009). A significance score of 10^{-5} was used as a cut-off value for inclusion in the list of candidate genes. The Integrated Complex Traits Networks interface (iCTNet), can be accessed through the Cytoscape plugin (Wang, et al., 2011). This database allows the automated construction of disease networks and integrates

phenotype-SNP, protein-protein interaction, disease-tissue, tissue-gene and drug-gene interactions. We queried the biological networks associated with PD and T2DM using a cutoff p-value of 10^{-5}. Genetic associations obtained from the aforementioned databases were manually curated after searching the literature in Pubmed. Functional and gene ontology analysis was performed using GENEMANIA plugin in Cytoscape (Montojo, et al., 2010). We used the default settings of 20 additional connecting genes and advanced settings to include only physical, predicted, genetic interactions, and interconnected pathways.

2.2 Gene prioritization methods and cross-validation analysis

Gene prioritization and cross validation analysis were performed using GPEC, a Cytoscape 2.8.3 plugin that performs a random walk-based algorithm. We used the default, weighted and undirected human FLN for this analysis. Genes known to be associated with PD and T2DM were retrieved from the OMIM (http://www.ncbi.nlm.nih.gov/omim), the Genetic association database (GAD) (http://geneticassociationdb.nih.gov/) and PDgene (http://www.pdgene.org/) (Supplementary Table 2). Genes involved in the PD and T2DM signaling pathways were retrieved from the KEGG database (http://www.genome.jp/kegg/pathway.html). To quantify the predictive capacity between PD and T2DM, we first used genes that are associated with PD as a training set (Supplementary Table 2). The candidate set included the cluster of 85 genes shared between both diseases and genes associated with T2DM as a background. To perform the RWR, we set back-probability to 0.5 and candidate genes were scored and ranked. Prioritization with respect to the biological pathways disrupted

in both diseases was performed for each individual pathway. For this purpose, we collected the set of genes curated for each biological pathway from the Broad Institute's Molecular Signatures Database (MSigDB) 3.0 (Subramanian, et al., 2005)(Supplementary Table 2). In this approach, the training set consisted of genes curated for each pathway disrupted in PD and T2DM and the test set consisted of the 85 genes in the shared cluster. A ROC curve of sensitivity versus 1-specificity was built to evaluate the performance of each prioritization. The final score for each gene was defined as the sum of all individual scores obtained from each prioritization.

2.3. Information about HBS and PROBE study participants

The Institutional Review Boards of Rosalind Franklin University of Medicine and Science approved the study protocol. Written informed consent was received from all participants. We used 96 individuals including 50 PD patients (31 men, 19 women; Hoehn and Yahr scale 1.97±0.62; mean age at enrollment 63.12±8.96; mean age at onset 58.75±10.17) and 46 healthy age-matched controls (26 men, 20 women; mean age at enrollment 64.28±10.42) enrolled in the HBS. Other clinical information is reported in (Santiago and Potashkin, 2013a). Details of patient and controls recruitment, clinical assessments, and biobanking in the HBS study population have been reported in part elsewhere (Ding, et al., 2011) and http://www.neurodiscovery.harvard.edu/research/biomarkers.html. As an independent replication set, we used 51 PD patients (29 men, 22 women; mean age at enrollment 63.16±6.42; Hoehn and Yahr scale 2±0.28) and 45 healthy age-matched controls (24 male, 21 women; mean age at enrollment 65.12±8.60) enrolled in the PROBE Study

(#NCT00653783). Clinical diagnosis of PD was based on the United Kingdom Parkinson's Disease Society Brain Bank criteria. Healthy controls had no history of neurological disease and a Mini-Mental State Examination (MMSE) test score higher than 27. Inclusion and exclusion criteria for patients enrolled in the PROBE study are reported elsewhere in (Potashkin, et al., 2012).

2.4. RNA isolation and real time polymerase chain reactions

Blood was collected and prepared as described using the PAXgene Blood RNA system (Qiagen,Valencia, CA) (Scherzer, et al., 2007). Samples with RNA integrity values > 7.0 and ratio of absorbances at 260/280 nm between 1.7 and 2.4 were used in the current study. Primer Express software (Life Technologies, Carlsbad, CA, USA) was used to design the primers. The High Capacity RNA transcription kit (Life Technologies, Carlsbad, CA) was used to reverse transcribe 1 μg of total RNA according to the manufacturer's protocol. The DNA engine Opticon 2 Analyzer (Bio-Rad Life Sciences, Hercules, CA, USA) was used for the qPCR reactions. Each 25 μl reaction contained Power SYBR (Life Technologies, Carlsbad, CA, USA) and primers at a concentration of 5 μM. Primer sequences used in qPCR assays are as follows: *GAPDH*; forward: 5'-CAACGGATTTGGTCGTATTGG-3'; reverse: 5'-TGATGGCAACAATATCCACTTTACC-3', *HNF4A*; forward: 5'-CAGAATGAGCGGGACCGGATC-3'; reverse: 5'-CAGCAGCTGCTCCTTCATGGAC-3', *SOD2*; forward: 5'-GTTCAATGGTGGTGGTCATATCA-3'; reverse: 5'- GCAACTCCCCTTTGGGTTCT-

3'. Amplification conditions and detailed description of qPCR experiments are described in (Santiago and Potashkin, 2013a).

2.5. Statistical analysis

All analyses were performed with Prism4.0 (GraphPad, La Jolla, CA, USA) and Statistica 8.0 (Statsoft, OK, Tulsa, USA). A student t-test (two-tailed) was used to estimate the significance between PD cases and controls for numerical variables. Linear regression and Pearson correlation analysis was used to determine statistical significance for each biomarker adjusting for sex, age, Hoehn & Yahr scale in both cohorts and BMI in the HBS study. A ROC curve analysis was used to evaluate the diagnostic accuracy for each biomarker. A p-value less than 0.05 was regarded statistically significant.

3. Results

3.1. Shared susceptibility genes in PD and T2DM

We explored the DisGeNET database, a comprehensive database of the human genetic associations (Bauer-Mehren, et al., 2011). We queried the gene networks associated with both PD and T2DM. Analysis of the merged network revealed a cluster consisting of 41 shared genetic connections between PD and T2DM including *BDNF*, *DRD2*, *ATF6*, and *NOS2* (Supplementary Table 1, Methods). Review of the literature confirmed existing associations between this group of genes with both diseases. For example, *BDNF* mRNA expression is reduced in the substantia nigra of PD (Howells, et al., 2000) and in blood of T2DM patients (Krabbe, et al., 2007). Polymorphisms of *DRD2* have been associated with PD susceptibility (Kiyohara, et al., 2011). In the context of diabetes, disruption of

the *DRD2* receptor impairs insulin secretion and causes glucose intolerance in mice (Garcia-Tornadu, et al., 2010).

Interestingly, several genes found in the shared cluster, including *ATF6* and *NOS2*, are associated with pathways disrupted in both diseases. It is well documented that endoplasmic reticulum (ER) stress is involved in the pathogenesis of PD and T2DM. In the context of PD, growing evidence indicates a functional contribution of ER stress to neurodegeneration and targeting components of the unfolded protein response pathway (UPR) may be useful therapeutically (Mercado, et al., 2013). Accordingly, ATF6 transcription factor that activates target genes for the UPR during ER stress, is neuroprotective in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD (Egawa, et al., 2011). With regards to prevention and promotion of diabetes, it has been suggested that ATF6α may protect pancreatic beta cells from ER stress and play a role in insulin resistance (Usui, et al., 2012). In addition to ER stress, oxidative stress is well known to contribute to neurodegeneration in PD. Nitric oxide synthase genes including *NOS2* are implicated in increased risk of PD (Hancock, et al., 2008) and T2DM (Bagarolli, et al., 2010).

We next explored the DGA interface (Peng, et al., 2013) and collected a group of 20 genes associated with PD and T2DM in addition to the set of genes found in DisGeNET (Supplementary Table 1). Genes involved in insulin signaling including *AKT1*, *IGF1*, and *E2F1* were present in this database. For example, genetic variations in *AKT* are implicated in glucose homeostasis and T2DM (Hay, 2011) and PD susceptibility in some

populations (Xiromerisiou, et al., 2008). Similarly, *E2F1* is involved in a regulatory pathway that controls insulin secretion (Annicotte, et al., 2009) and is suggested to be a key mediator of cell death in PD (Hoglinger, et al., 2007).

We also interrogated the HEFalMp interface (Huttenhower, et al., 2009). Similarly to DGA and DisGeNeT, we investigated the genetic associations between PD and T2DM. The most significant genes in T2DM associated to PD were *HNF4A*, *PDX1*, *SLC2A4*, and *ABCC8* ($Q< 10^{-05}$)(Supplementary Table 1).

Finally, we explored the iCTNet interface (Wang, et al., 2011) that contains results from 118 GWAS published studies and data from the GWAS catalog. Gene network analysis revealed a set containing 20 genes shared between both diseases (Supplementary Table 1**).** Several interesting genes found in this shared cluster included *PPARG*, *PPARGC1A*, and *HNF4A*. Not surprisingly, this group of genes has been associated with PD and T2DM. The peroxisome proliferator activator receptor gamma (PPAR-γ) and PPAR-γ coactivator-1α (PGC-1α) have been studied as potential therapeutic targets in PD (Schintu, et al., 2009,St-Pierre, et al., 2006,Zheng, et al., 2010) and T2DM (Han, et al., 2008) given its involvement in inflammation and lipid signaling (Wahli and Michalik, 2012). HNF4α may also play a role in intestinal lipid metabolism, oxidative stress and inflammation, processes that are implicated in both chronic diseases (Marcil, et al., 2010).

3.2. Biological and functional analysis

To further identify the potential functional implications in the cluster of genes shared between PD and T2DM, we imported all 85 genes into GeneMANIA (Montojo, et al., 2010). Analysis of the PD-T2DM cluster using GeneMANIA identified the most overrepresented pathways including nitric oxide biosynthetic processing, carbohydrate and lipid metabolic processing, insulin secretion, regulation of glucose, and inflammation (Figure 2).

3.3. Gene prioritization and experimental validation

Given the numerous molecular links between PD and T2DM, we investigated the extent to which genes identified within common dysregulated pathways can be used to classify patients with PD. We implemented a candidate prioritization approach using a RWR algorithm within the human the FLN described previously (Kohler, et al., 2008,Santiago and Potashkin, 2013a,Santiago and Potashkin, 2013b). This algorithm measures the closeness of potentially candidate genes to confirmed genes within the FLN or protein-protein interaction network. We used GPEC, a cytoscape plugin for random walk-based gene prioritization (Le and Kwon, 2012) to rank 85 candidates collected from the curated databases (Supplementary Table 1). In the RWR algorithm, the known disease genes are mapped to the FLN and specified as “training set” and the “test set” containing potential candidates can be ranked according to their closeness to the training genes within the FLN (See Methods). RWR score-based genes are listed in Supplementary Table 3. Further, we evaluated the performance of the gene prioritization using a leave-one-out cross validation (LOOCV) strategy. LOOCV represented in terms of receiver operating characteristic curve (ROC) resulted in an area under curve $AUC_{PD\text{-}T2DM}$ value of 0.85

(Supplementary figure 1A). The AUC value is an estimate of the predictive capacity, where an AUC value of 1 is indicative of high predictability and an AUC value of less than 0.5 indicates poor predictability and random distribution.

As a second step, we prioritized genes with respect to the biological pathways disrupted in both diseases. We collected the set of genes curated for each biological pathway from the Broad Institute's Molecular Signatures Database 3.0 (MSigDB) (Subramanian, et al., 2005)(Supplementary Table 2). Gene prioritization was performed for each individual pathway (Materials and Methods, Supplementary Table 3). LOOCV performed for each prioritization resulted in AUC values ranging from 0.90-0.99 (Supplementary figure 1B-E). The top RWR score-based genes are listed in Table 1. The complete list of RWR score-based candidate genes according to each prioritization step is provided in (Supplementary Table 3).

In order to validate the results obtained from the network analysis we evaluated highly ranked genes, *HNF4A* and *SOD2,* as potential biomarkers for PD. Relative abundance of these biomarkers was measured in whole blood of PD patients compared to healthy individuals from samples obtained from two independent clinical trials, HBS and PROBE. Quantitative PCR assays revealed that *SOD2* mRNA and *HNF4A* mRNA are significantly upregulated in blood of PD patients compared to HC in both cohorts of study participants, although significant overlap in expression levels of both biomarkers was observed between PD and controls (Figure 3A and B). To evaluate the diagnostic accuracy of *HNF4A* and *SOD2* in distinguishing PD patients from HC, ROC curve

analysis was performed. As shown in Figure 3C and D, the AUC values for *HNF4A* and *SOD2* were 0.72 and 0.69, respectively. Combination of both biomarkers in the analysis did not improve the diagnostic accuracy.

Pearson correlation analysis demonstrated that relative abundance of each biomarker was independent of other covariates including age (*SOD2*: r=-0.13, p=0.40, *HNF4A:* r=-0.25, p=0.9), and sex (*SOD2*: r=-0.03, p=0.79, *HNF4A:* r=-0.004, p=0.97) in both cohorts of patients and BMI (*SOD2*: r=0.18, p=0.21, *HNF4A*: r=-0.005, p=0.96) in the HBS cohort. Correlation analysis revealed a significant negative correlation between *HNF4A* mRNA expression and Hoehn and Yahr stage (r= -0.29, p=0.009, Figure 4), but not significant for *SOD2* mRNA (r=0.04, p=0.73). Correlation of relative abundance of each biomarker with medication was not determined since most of the patients with PD were medicated with several drugs and the number of untreated patients was too small to reliably detect a significant change.

4. Discussion

Integration of networks generated from multiple disease-gene databases revealed a molecular cluster comprising 85 genes shared between PD and T2DM. Biological and functional analysis of the PD-T2DM cluster identified shared dysregulated pathways including nitric oxide biosynthesis, regulation of glucose, lipid and carbohydrate metabolism, insulin secretion and inflammation. Recently, dysregulation of glucose metabolism was identified as an early event in sporadic PD and it has been hypothesized that SNCA may play a role in this process (Dunn, et al., 2014). In addition, metabolic

inflammation exacerbated dopaminergic cell death in diabetic mice exposed to MPTP (Wang, et al., 2014). These findings are consistent with previous studies that highlight the significant convergence of dysregulated pathways in PD and T2DM (Menon and Farina, 2011,Santiago and Potashkin, 2013a,Santiago and Potashkin, 2013c).

We further evaluated highly ranked genes, *HNF4A* and *SOD2* in blood of patients with PD from two independent cohorts of study participants. Gene expression levels of these biomarkers were upregulated in blood of PD patients compared to healthy individuals. We initially identified *HNF4A* as a putative regulator of PD risk markers (Potashkin, et al., 2012). Dysregulation of *HNF4A* in blood of PD patients is interesting given its role as a central metabolic regulator in gluconeogenesis, lipid and fatty acids. Gluconeogenesis is highly regulated by the interaction of HNF4A with PGC-1α and FOXO1 (Rhee, et al., 2003), factors implicated in PD. For example, repression of PGC-1α by PARIS (ZNF746), a parkin substrate, leads to the selective loss of dopamine neurons in PD (Shin, et al., 2011). Gene expression profiling of prefrontal cortex of PD patients revealed the upregulation of transcription factor FOXO1 and genes under its control (Dumitriu, et al., 2012). Therefore, the interaction of PGC-1α with HNF4α in the context of neurodegeneration warrants further investigation. More importantly, we found a significant negative correlation between *HNF4A* mRNA expression and the Hoehn and Yahr scale. PD patients with a low Hoehn and Yahr scale rating (HY=1) showed a significantly greater upregulation of *HNF4A* mRNA compared to patients with a higher Hoehn and Yahr scale. This finding suggests that *HNF4A* mRNA may be useful to identify patients at very early stages of PD when therapeutic intervention may be useful.

Further, *HNF4A* mRNA may be a useful biomarker to track the progression of PD. Follow-up studies are needed to evaluate the potential of *HNF4A* to identify patients at risk and to monitor disease progression.

In the same context, gene expression level of *SOD2* mRNA was upregulated in blood of PD patients. Superoxide dismutase 2 (*SOD2*) is a mitochondrial enzyme that protects against oxidative stress by converting superoxide radicals to molecular oxygen and hydrogen peroxide. Given its antioxidant capacity, it has been implicated in the pathogenesis of PD. For example, inactivation of SOD2 increases mitochondrial ROS production in *in vitro* models of PD (Belluzzi, et al., 2012). Moreover, SOD2 protein levels are increased in the frontal cortex of PD patients (Ferrer, et al., 2007).

Not surprisingly, *SOD2* and *HNF4A* have been extensively associated with diabetes. For example, genetic variations in *HNF4A* have been associated with disease susceptibility to diabetes (Gupta and Kaestner, 2004,Silander, et al., 2004) and increased levels of *SOD2* mRNA have been found in skeletal muscle of patients with T2DM (Reyna, et al., 2008).

Recently, drugs to treat diabetic patients, metformin-sulfonylurea and exenatide have shown promise in PD patients (Aviles-Olmos, et al., 2013,Wahlqvist, et al., 2012). Interestingly, metformin treatment results in decreased expression of *HNF4A* mRNA in primary rat hepatocytes (Lauer, et al., 2009) and increased expression of *SOD2* mRNA in human endothelial cells (Kukidome, et al., 2006). Troglitazone treatment, another anti-diabetic and anti-inflammatory drug, results in decreased expression of *HNF4A* and

SOD2 mRNAs in cellular models (Lauer, et al., 2009,Ruan, et al., 2003). In addition, gliclazide treatment, an oral sulfonylurea hypoglycemic agent, results in decreased protein expression of SOD2 (Onozato, et al., 2004), and rosiglitazone, an insulin sensitizer, increased SOD2 protein expression in retinal cells from mice (Doonan, et al., 2009). Based on these observations, expression of *HNF4A* and *SOD2* in blood may be useful to evaluate the therapeutic effect of anti-diabetic drugs in PD patients.

This study has several strengths and limitations. Biomarkers obtained from microarray studies may be data set specific and not indicative of the underlying disease pathology. In this context, our integrated network approach provides a framework to identify and prioritize PD biomarkers involved in common dysregulated pathways. Another strength is the replication of these biomarkers in two independent cohorts of patients. However, there are several limitations and potential confounding factors. For example, although we have found that *GAPDH* mRNA expression in blood is stable in previous studies (Potashkin, et al., 2012,Santiago and Potashkin, 2013a,Santiago and Potashkin, 2013b,Santiago, et al., 2013), replication of these biomarkers using several reference genes for normalization is desirable (Stamova, et al., 2009). In addition, differences in blood counts and PD medications may bias gene expression results. Thus, evaluation of these biomarkers in drug-naïve PD patients and in a large well-characterized prospective study will be important to determine the clinical utility of these biomarkers.

In summary, our study demonstrates that integration of shared molecular networks provides a useful framework to prioritize candidate biomarkers in a biologically relevant

context. Remarkably, we demonstrate that expression of genes identified within shared dysregulated pathways can be used as diagnostic markers for PD. We foresee integrated network approaches will provide a better understanding of the underlying disease mechanism and facilitate the discovery of accurate biomarkers and therapeutic targets. In this regard, a network-based approach was useful to identify a neuroprotective agent, alvespimycin (17-DMAG), in PD (Gao, et al., 2013). Future studies will be aimed to study the potential functional role of these biomarkers in PD.

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Disclosure Statement

Dr. Scherzer has collaborated with DiaGenic, Pfizer, Opko, and Proteome Sciences.

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Figures legends:

Figure 1. Integrative network approach. Shared genetic connections between PD and T2DM (yellow circles) were collected from multiple databases and mapped to the human FLN (black). Similarly, genes known to be associated with PD (purple circles) and T2DM (magenta circles) were mapped to the FLN and specified as training set. A random walk algorithm with restart (RWR) was implemented to prioritize candidate genes according to their distance to known disease genes and in terms of biological pathways involved. Highly ranked genes were evaluated as diagnostic biomarkers for PD on RNA samples from whole blood obtained from two independent clinical trials.

Figure 2. Biological functional analysis of candidate genes. Network of interactions among PD and T2DM related genes, as retrieved by GeneMANIA. Candidate genes are displayed in yellow circles and other genes connected to the candidate genes are displayed in gray circles. The size of the gray nodes represents the degree of association with the input genes (i.e., smaller size represents low connectivity). The most represented pathways retrieved by GeneMANIA are displayed using GO annotations and Q-values of significance.

Figure 3. Evaluation of *HNF4A* and *SOD2* as potential biomarkers for PD.

A. Relative abundance of biomarkers in blood of PD patients (black circles) compared to healthy controls (white circles) in samples from the HBS cohort. **B.** Replication of biomarker expression in an independent set of samples from patients enrolled in the PROBE study. Relative abundance of each biomarker was calculated using *GAPDH* as a reference gene and healthy controls as calibrator. Error bars represent standard error. **C.** ROC curve analysis to evaluate the diagnostic accuracy of *HNF4A* and D. ROC curve analysis to evaluate the diagnostic accuracy of *SOD2.*

Figure 4. Correlation of *HNF4A* mRNA with Hoehn and Yahr stage. Correlation analysis of *HNF4A* mRNA expression and Hoehn and Yahr stage in both cohorts of patients. Error bars represent standard error.

Tables:

Table 1. Highly ranked RWR score-based genes.

Rank	Gene Symbol	Gene Name	Score
1	*SOD2*	Superoxide dismutase 2	3.08E-03
2	*MT-ND1*	Mytochondrially encoded NADH dehydrogenase 1	2.93E-03
3	*IFNG*	Interferon, gamma	2.90E-03
4	*TNF*	Tumor necrosis factor	2.39E-03
5	*TP53*	Tumor protein p53	2.36E-03
6	*IL6*	Interleukin 6	2.16E-03
7	*AKT1*	V-akt murine thymoma viral oncogene homolog 1	1.96E-03
8	*HNF4A*	Hepatocyte nuclear factor 4, alpha	1.80E-03
9	*HMOX1*	Heme oxygenase (decycling) 1	1.77E-03
10	*FAS*	Fas (TNF receptor superfamily, member 6)	1.53E-03
11	*APP*	Amyloid beta (A4) precursor protein	1.34E-03
12	*CYP17A1*	Cytochrome P450, family 17, subfamily A, polypeptide 1	1.23E-03
13	*IGF1*	Insulin-like growth factor 1	1.03E-03
14	*PTGS2*	Prostaglandin-endoperoxide synthase 2	1.02E-03
15	*SOD1*	Superoxide dismutase 1, soluble	9.80E-04
16	*BDNF*	Brain-derived neurotrophic factor	8.46E-04
17	*NOS2*	Nitric oxide synthase 2	8.34E-04
18	*TGM2*	Transglutaminase 2	6.86E-04
19	*GCH1*	GTP cyclohydrolase 1	6.66E-04
20	*UCHL1*	Ubiquitin carboxyl-terminal esterase L1	6.60E-04

Supplementary figures:

Supplementary Figure 1. Validation of each prioritization step. The performance of each prioritization step was validated by computing values for ROC and AUC through the leave-one-out validation method using GPEC.

Supplementary tables:

Supplementary Table 1. PD and T2DM shared cluster of genes.

Gene Symbol	Entrez ID	Database
VDR	7421	DisGeNET
TNF	7124	DisGeNET
TH	7054	DisGeNET
TF	7018	DisGeNET
SOD2	6648	DisGeNET
PTGS2	5743	DisGeNET
PON1	5444	DisGeNET
PINK1	65018	DisGeNET
PARP1	142	DisGeNET
OPRM1	4988	DisGeNET
NQO1	1728	DisGeNET
NPPB	4879	DisGeNET
NOS2	4843	DisGeNET
NFKB1	4790	DisGeNET
MTND1	4535	DisGeNET
NAT2	10	DisGeNET
MTHFR	4524	DisGeNET

INS	3630	DisGeNET
IL6	3569	DisGeNET
IL1B	3553	DisGeNET
IGF2	3481	DisGeNET
IFNG	3458	DisGeNET
IDE	3416	DisGeNET
HP	3240	DisGeNET
HMOX1	3162	DisGeNET
HGF	3082	DisGeNET
HFE	3077	DisGeNET
GPX1	2876	DisGeNET
FAS	355	DisGeNET
DRD2	1813	DisGeNET
CYP1A1	1543	DisGeNET
CYP17A1	1586	DisGeNET
CDKN2A	1029	DisGeNET
CD14	929	DisGeNET
CCL5	6352	DisGeNET
CCL2	6347	DisGeNET
BDNF	627	DisGeNET
ATF6	22926	DisGeNET
APOE	348	DisGeNET
ADH1C	126	DisGeNET
ACE	1636	DisGeNET
ABCB1	5243	DGA
AKT1	207	DGA
APP	351	DGA

BCHE	590	DGA
CP	1356	DGA
E2F1	1869	DGA
GAD1	2571	DGA
GAD2	2572	DGA
GCH1	2643	DGA
GH1	2688	DGA
GSTM1	2944	DGA
IGF1	3479	DGA
IL8	3576	DGA
MAOB	4129	DGA
SLC18A2	6571	DGA
SOD1	6647	DGA
TGM2	7052	DGA
TP53	7157	DGA
TSC2	7249	DGA
UCHL1	7345	DGA
BTG1	694	iCTNet
HNF4A	3172	iCTNet
SEMA6A	57556	iCTNet
CXCR4	7852	iCTNet
RBMS3	27303	iCTNet
PPARG	5468	iCTNet
HNF1A	6927	iCTNet
TCF7L2	6934	iCTNet
TBC1D22A	25771	iCTNet
OLFM4	10562	iCTNet

SORBS1	10580	iCTNet
CADM1	23705	iCTNet
PCDH18	54510	iCTNet
NCAM2	4685	iCTNet
MMP16	4325	iCTNet
SERPINB1	1992	iCTNet
PPARGC1A	10891	iCTNet
MBNL1	4154	iCTNet
KIF11	3832	iCTNet
KCNJ2	3759	iCTNet
CXCL12	6387	iCTNet
PDX1	3651	HEFalMp
SLC2A4	6517	HEFalMp
ABCC8	6833	HEFalMp

Supplementary Table 2. Curated gene sets used for RWR prioritization.

Disease or biological pathway	Gene sets
Parkinson's disease	KEGG 05012: PD signaling pathway PDgene, GAD, OMIM: *GAK, DGKQ, STH, MAPT, LRRK2, SNCA, LOC642072, WNT3, RIT2, GBA, MCCC1, LAMP3, SCARB2, SYT11, ACMSD, STK39, BST1, HLA-DRB5, CCDC62, HIP1R, HLA-DRA, PARK16, SLC45A3, NUCKS1, RAB7L1, SLC41A1, PM20D1, C17ORF69, KIAA1267, LOC644246, NSF, FAM47E, SREBF1, TMEM175, BRDG1, DLG2, PLEKHM1, IMP5, CRHR1, PM20D1*
Type 2 diabetes	KEGG 04930: T2DM signaling pathway GAD and OMIM: *ARF5, PAX4, SND1, IGF2BP2,* *GRK5, RASGRP1, GLIS3, CDKN2B, CDC123, HNF1B, FAM58A, DUSP9, CDKAL1, LAMA1, FTO, HHEX, RBM43, RND3, MAEA, GLIS3, FITM2, R3HDML, GCC1, PSMD6, ZFAND3, HMG20A, AP3S2, KCNQ1, SPRY2, C2CD4A, C2CD4B, BCL11A, ZBED3, KLF14, TP53INP1, CENTD2, HMGA2, ZFAND6, PRC1, IRS1, MTNR1B, JAZF1, IDE, SRR, PTPRD, SLC30A8, CAMK1D, TSPAN8, LGR5, THADA*
Insulin signaling	KEGG: 04910
Nitric oxide biosynthesis	MSigDB: M11650 BioCarta: Nitric oxide signaling pathway
Glucose metabolism	MSigDB: M1879 Reactome glucose metabolism
Inflammation	KEGG: 04062
Lipid metabolism	KEGG: 00071

Supplementary Table 3. RWR-based scores for each prioritization within the functional linkage network. Score PD-T2DM are the scores for the disease prioritization, p1 is insulin signaling pathway, p2 is nitric oxide biosynthesis, p3 is glucose metabolism, p4 is inflammation, p5 is lipid metabolism and c is the cumulative score.

Rank	Gene	Score (PD-T2DM)	Score (p1)	Score (p2)	Score (p3)	Score (p4)	Score (p5)	Score (c)
1	*SOD2*	9.07E-04	1.49E-04	2.21E-04	1.37E-03	3.73E-05	3.96E-04	3.08E-03
2	*MTND1*	2.68E-03	1.41E-05	1.16E-05	1.47E-04	1.94E-06	7.71E-05	2.93E-03
3	*TNF*	2.18E-04	4.75E-04	1.38E-04	2.46E-04	1.76E-03	5.96E-05	2.90E-03
4	*IFNG*	7.27E-05	2.99E-04	7.12E-05	5.00E-05	1.88E-03	2.39E-05	2.39E-03
5	*TP53*	5.18E-04	5.58E-04	2.70E-04	3.90E-04	3.96E-04	2.26E-04	2.36E-03
6	*IL6*	7.38E-05	3.67E-04	1.01E-04	5.93E-05	1.53E-03	3.17E-05	2.16E-03
7	*AKT1*	2.10E-04	1.20E-03	5.16E-04	0.00E+00	0.00E+00	3.20E-05	1.96E-03
8	*HNF4A*	4.02E-05	6.02E-04	2.89E-05	7.33E-04	2.42E-05	3.74E-04	1.80E-03
9	*HMOX1*	1.47E-04	5.53E-05	3.04E-04	4.63E-05	3.48E-05	1.18E-03	1.77E-03
10	*FAS*	1.87E-04	2.52E-04	6.25E-04	1.70E-04	2.69E-04	3.20E-05	1.53E-03
11	*APP*	3.17E-04	4.73E-04	1.39E-04	1.17E-04	1.97E-04	9.77E-05	1.34E-03
12	*CYP17A1*	2.99E-05	4.50E-05	2.06E-04	2.97E-05	4.16E-05	8.76E-04	1.23E-03
13	*IGF1*	3.50E-05	4.96E-04	6.35E-05	5.54E-05	3.60E-04	1.53E-05	1.03E-03
14	*PTGS2*	1.40E-04	2.16E-04	2.90E-04	2.70E-05	2.05E-04	1.39E-04	1.02E-03
15	*SOD1*	2.89E-04	2.55E-05	2.22E-04	2.10E-04	2.70E-05	2.07E-04	9.80E-04
16	*BDNF*	1.97E-05	5.17E-05	6.01E-04	2.24E-05	1.01E-04	5.04E-05	8.46E-04
17	*NOS2*	3.27E-05	5.20E-05	4.59E-04	2.52E-05	1.45E-04	1.21E-04	8.34E-04
18	*TGM2*	2.12E-05	9.00E-05	3.31E-05	7.09E-06	3.18E-05	5.03E-04	6.86E-04
19	*GCH1*	1.48E-04	4.13E-05	9.22E-06	2.58E-04	4.14E-06	2.06E-04	6.66E-04
20	*UCHL1*	1.29E-04	7.90E-07	6.00E-07	5.28E-04	9.60E-07	1.30E-07	6.60E-04
21	*IL1B*	2.90E-05	5.13E-05	2.85E-05	2.10E-05	5.10E-04	7.69E-06	6.48E-04
22	*HNF1A*	1.88E-05	1.35E-04	1.69E-05	2.00E-04	3.02E-05	1.81E-04	5.82E-04
23	*APOE*	5.10E-05	1.28E-04	6.59E-05	4.33E-05	2.45E-04	1.91E-05	5.52E-04
24	*IGF2*	2.88E-05	2.32E-04	2.61E-05	1.65E-04	6.39E-05	1.35E-05	5.29E-04

25	*CYP1A1*	1.42E-05	1.04E-05	3.93E-05	1.18E-05	5.55E-06	4.47E-04	5.28E-04
26	*PPARG*	7.15E-05	2.07E-04	1.79E-05	1.30E-04	7.45E-05	1.56E-05	5.16E-04
27	*SLC18A2*	4.16E-04	4.01E-05	2.72E-05	8.78E-06	5.75E-06	6.66E-06	5.05E-04
28	*CD14*	2.29E-04	3.94E-05	1.91E-05	1.14E-05	1.96E-04	6.13E-06	5.01E-04
29	*PINK1*	9.85E-05	1.25E-04	8.76E-05	5.50E-05	8.71E-05	2.01E-06	4.55E-04
30	*INS*	8.21E-05	0.00E+00	7.97E-05	1.30E-04	1.00E-04	1.88E-05	4.10E-04
31	*PARP1*	1.81E-04	7.52E-05	3.56E-05	2.93E-05	5.59E-05	1.60E-05	3.93E-04
32	*NFKB1*	1.62E-04	1.34E-04	5.60E-05	3.34E-05	0.00E+00	6.88E-06	3.92E-04
33	*SLC2A4*	1.88E-04	0.00E+00	1.59E-05	6.06E-05	1.55E-05	9.41E-05	3.74E-04
34	*IDE*	9.91E-05	7.13E-05	1.21E-05	5.38E-05	1.49E-05	1.21E-04	3.72E-04
36	*DRD2*	1.09E-04	4.91E-05	6.36E-05	1.56E-05	1.20E-04	8.04E-06	3.66E-04
37	*GAD2*	1.79E-05	7.99E-06	1.68E-05	2.67E-04	1.21E-05	2.78E-05	3.50E-04
38	*SORBS1*	3.21E-05	0.00E+00	1.04E-04	1.30E-05	1.96E-04	4.86E-06	3.50E-04
39	*CP*	1.43E-04	1.61E-05	2.32E-05	2.32E-05	1.19E-05	1.18E-04	3.35E-04
40	*TH*	1.64E-04	5.27E-06	3.49E-06	1.32E-04	2.75E-06	8.15E-06	3.16E-04
41	*TSC2*	1.53E-05	0.00E+00	3.85E-05	6.54E-05	1.80E-04	3.69E-06	3.02E-04
42	*PON1*	7.71E-06	8.29E-05	1.48E-05	1.73E-04	5.60E-06	7.00E-06	2.91E-04
35	*E2F1*	3.08E-05	1.17E-04	1.95E-05	3.01E-05	8.21E-05	5.79E-06	2.85E-04
43	*CXCR4*	3.42E-05	1.74E-04	4.56E-05	1.89E-05	0.00E+00	5.69E-06	2.78E-04
44	*CDKN2A*	4.95E-05	8.19E-05	3.34E-05	2.77E-05	5.81E-05	7.88E-06	2.59E-04
45	*KCNJ2*	3.68E-05	9.63E-06	1.80E-04	4.50E-06	7.34E-06	8.90E-07	2.39E-04
46	*PPARGC1A*	8.59E-05	0.00E+00	1.60E-05	9.91E-05	1.93E-05	1.05E-05	2.31E-04
47	*HGF*	1.67E-05	4.80E-05	2.10E-05	1.04E-05	8.99E-05	8.56E-06	1.95E-04
48	*OPRM1*	6.93E-05	1.64E-05	3.46E-05	3.93E-06	6.48E-05	5.90E-07	1.90E-04
49	*TF*	4.58E-05	3.04E-05	2.36E-05	2.87E-05	2.40E-05	2.41E-05	1.77E-04
50	*ACE*	2.33E-05	2.06E-05	2.16E-05	3.08E-05	6.03E-05	1.68E-05	1.73E-04
51	*CADM1*	8.86E-06	4.13E-05	8.41E-05	4.55E-06	3.34E-05	1.18E-06	1.73E-04
52	*NQO1*	2.52E-05	4.81E-06	8.78E-06	5.38E-05	1.76E-06	3.06E-05	1.25E-04
53	*GAD1*	1.45E-05	5.59E-06	1.94E-05	4.08E-05	1.15E-06	4.07E-05	1.22E-04
54	*GH1*	4.26E-06	6.78E-05	6.50E-06	1.13E-05	2.16E-05	3.76E-06	1.15E-04

55	*HFE*	6.71E-05	7.37E-06	6.62E-06	1.02E-05	8.94E-06	1.20E-05	1.12E-04
56	*CXCL12*	1.68E-05	4.53E-05	2.75E-05	1.38E-05	0.00E+00	5.87E-06	1.09E-04
57	*ABCB1*	2.46E-05	1.12E-05	8.73E-06	9.51E-06	4.93E-06	3.38E-05	9.27E-05
58	*MAOB*	2.62E-05	4.99E-06	6.56E-06	1.26E-05	2.86E-06	3.83E-05	9.15E-05
59	*BTG1*	4.42E-06	2.90E-05	8.75E-06	3.89E-06	3.93E-05	1.25E-06	8.66E-05
60	*ABCC8*	4.46E-06	2.51E-05	3.57E-05	9.80E-06	2.23E-06	8.63E-06	8.58E-05
61	*PDX1*	6.06E-06	3.74E-05	8.36E-06	1.61E-05	9.14E-06	4.64E-06	8.16E-05
62	*ADH1C*	7.61E-06	3.92E-05	7.80E-07	3.25E-05	3.70E-07	0.00E+00	8.04E-05
63	*CCL5*	1.44E-05	3.18E-05	1.91E-05	8.35E-06	0.00E+00	2.33E-06	7.60E-05
64	*TCF7L2*	1.13E-05	2.25E-05	9.84E-06	8.55E-06	1.77E-05	3.25E-06	7.32E-05
65	*ATF6*	1.62E-05	1.71E-05	1.00E-05	6.65E-06	1.39E-05	2.80E-06	6.66E-05
66	*GPX1*	3.84E-05	2.84E-06	2.18E-06	1.00E-05	1.50E-06	1.06E-05	6.56E-05
67	*CCL2*	1.04E-05	2.89E-05	1.42E-05	7.16E-06	0.00E+00	2.60E-06	6.32E-05
68	*VDR*	8.29E-06	1.41E-05	6.71E-06	8.37E-06	8.68E-06	1.56E-05	6.18E-05
69	*BCHE*	5.29E-06	1.31E-05	2.93E-05	5.56E-06	3.39E-06	4.89E-06	6.15E-05
70	*MTHFR*	1.12E-05	4.88E-06	1.88E-06	2.98E-05	2.32E-06	1.12E-05	6.12E-05
71	*IL8*	1.01E-05	2.55E-05	1.21E-05	1.00E-05	0.00E+00	2.76E-06	6.04E-05
72	*KIF11*	1.11E-05	1.34E-05	1.79E-05	7.16E-06	9.12E-06	1.18E-06	5.99E-05
73	*MMP16*	2.99E-06	4.76E-06	3.01E-06	3.24E-06	1.65E-05	1.51E-06	3.20E-05
74	*GSTM1*	1.11E-05	1.73E-06	1.33E-06	3.94E-06	5.70E-07	1.29E-05	3.16E-05
75	*HP*	5.81E-06	4.87E-06	3.74E-06	3.76E-06	8.82E-06	1.60E-06	2.86E-05
76	*NPPB*	8.80E-07	2.50E-06	6.81E-06	5.40E-07	1.64E-05	4.70E-07	2.76E-05
77	*SEMA6A*	5.00E-07	3.89E-06	2.06E-06	3.60E-07	4.98E-06	9.00E-08	1.19E-05
78	*NCAM2*	1.28E-06	2.10E-06	2.95E-06	3.50E-07	3.00E-06	1.30E-07	9.81E-06
79	*SERPINB1*	2.33E-06	1.53E-06	6.10E-07	1.59E-06	2.97E-06	5.20E-07	9.55E-06
80	*NAT2*	1.29E-06	2.90E-07	1.20E-07	2.30E-06	4.00E-08	1.46E-06	5.50E-06
81	*MBNL1*	4.50E-07	1.56E-06	8.20E-07	7.00E-07	6.30E-07	1.10E-07	4.27E-06
82	*PCDH18*	1.80E-07	1.51E-06	8.60E-07	1.10E-07	1.46E-06	4.00E-08	4.16E-06
83	*OLFM4*	8.00E-08	0.00E+00	1.80E-06	8.00E-08	8.00E-08	1.00E-08	2.05E-06
84	*RBMS3*	1.20E-07	2.50E-07	1.10E-07	1.37E-06	6.00E-08	6.00E-08	1.97E-06

85	*TBC1D22A*	2.30E-07	1.40E-07	4.10E-07	2.30E-07	5.00E-08	6.00E-08	1.12E-06

Clinical & Experimental Pharmacology

Editorial Open Access

Network Analysis Accelerates Understanding of Disease Mechanisms

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During the last decade, high throughput methods including gene profiling and genome wide association studies have identified thousands of genetic risk factors, biological pathways and biomarkers for a wide range of diseases. Despite this apparent success, the translation of this valuable data into clinical tools for disease diagnosis, prognosis and treatment remains challenging due to the rigorous and highly complex statistical methods used and the limited functional and biological information derived from these datasets. To address this hurdle, network biology has emerged as a powerful tool for the characterization of complex diseases by integrating genetic and environmental factors into a single system with biological relevance.

Based on the observation that causal genes of disease tend to be interconnected in common biological modules [1], the study of how disruption in these genetic networks lead to disease has been fundamental in the understanding of many complex diseases. In this regard, network biology has elucidated molecular networks causative of disease. For example, integration of co-expression networks and genotypic data identified networks associated with metabolic disease and causal genes for obesity [2,3]. An alternative method to discover novel causative disease genes is to explore the interconnectivity and distance of well-characterized genes to closely associated neighbors in a gene or protein interaction network. Using this strategy, novel susceptibility genes and underlying disease mechanisms were identified in Alzheimer's disease (AD) [4].

Another area of great interest is understanding disease comorbities. Network approaches have been useful in dissecting and providing insight into the underlying mechanism leading to concurrent diseases. Remarkably, analysis of the human metabolic network revealed that connected diseases with metabolic links displayed higher comorbidity than those with no metabolic links [5]. Given the intriguing hypothesis of a shared mechanisms leading to Parkinson's disease and diabetes [6], this approach could provide insights into this relationship.

Biomarker discovery is another area of promise in the field of network biology. There are several challenges in the discovery and validation of biomarkers from high-throughput studies. One problem arising is that biomarkers from microarray studies can be data set-specific and provide limited information about the underlying disease etiology. Also, the biomarker signature identified depends on the statistical approach used in the analysis. In addition, it has become evident that there is very little overlap between biomarker sets identified for a particular disease using similar gene profiling methods. To address these issues, network based approaches are expected to facilitate the integration of multiple lines of information to identify accurate and reliable biomarkers for disease diagnosis. In this context, network approaches have successfully identified biomarkers with clinical applicability. For example, using a well characterized set of genes, a network approach identified biomarkers for progressive supranuclear palsy [7]. This approach utilizes a random walk algorithm with restart in which a random walker moves from a known disease gene to a random neighbor within a specified distance in the functional linkage network [8]. Biosignatures for colorectal cancer were also identified using this approach [9].

In summary, network-based approaches provide an innovative framework to dissect complex diseases. With the emerging field of RNA-sequencing technology, the amount of data and information is expected to greatly increase; therefore, integrative network approaches will be valuable to dissect the relevant biological information. Based on the recent success in identifying biomarkers for disease diagnosis, we foresee that network-based approaches will facilitate the discovery of therapeutic targets. Further, we expect that networks and systems biology will accelerate the translation of biomarkers and therapeutics to the clinic.

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1. Oti M, Brunner HG (2007) The modular nature of genetic diseases. Clin Genet 71: 1-11.
2. Chen Y, Zhu J, Lum PY, Yang X, Pinto S, et al. (2008) Variations in DNA elucidate molecular networks that cause disease. Nature 452: 429-435.
3. Yang X, Deignan JL, Qi H, Zhu J, Qian S, et al. (2009) Validation of candidate causal genes for obesity that affect shared metabolic pathways and networks. Nat Genet 41: 415-423.
4. Soler-López M, Zanzoni A, Lluís R, Stelzl U, Aloy P (2011) Interactome mapping suggests new mechanistic details underlying Alzheimer's disease. Genome Res 21: 364-376.
5. Lee DS, Park J, Kay KA, Christakis NA, Oltvai ZN, et al. (2008) The implications of human metabolic network topology for disease comorbidity. Proc Natl Acad Sci U S A 105: 9880-9885.
6. Santiago JA, Potashkin JA (2013) Shared dysregulated pathways lead to Parkinson's disease and diabetes. Trends Mol Med 19: 176-186.
7. Santiago JA, Potashkin JA (in press) A Network Approach to Diagnostic Biomarkers in Progressive Supranuclear Palsy. Mov Disord.
8. Köhler S, Bauer S, Horn D, Robinson PN (2008) Walking the interactome for prioritization of candidate disease genes. Am J Hum Genet 82: 949-958.
9. Shi M, Beauchamp RD, Zhang B (2012) A network-based gene expression signature informs prognosis and treatment for colorectal cancer patients. PLoS

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Judith A. Potashkin

Department of Cellular and Molecular Pharmacology
Rosalind Franklin University of Medicine and Science
The Chicago Medical School

Degrees

1977	B.A., Summa cum laude, Biology Department Lehigh University, Bethlehem, PA. Field: Biology
1979	M.S., Department of Microbiology, Cell Biology, Biochemistry and Biophysics Pennsylvania State University, University Park, PA. Field: Cell Biology and Biochemistry Thesis: An investigation of a possible mechanism of stimulation of ribosomal ribonucleic acid sysnthesis by simian virus 40 T-antigen
1985	Ph.D., Department of Cell and Tumor Biology Roswell Park Memorial Institute, Buffalo, N.Y. Field: Molecular Biology Thesis: Isolation and characterization of residual nuclei from *Saccharomyces cerevisiae*

Training

9/76-5/77	Undergraduate trainee in parasitology Laboratory of Dr. Thomas Cheng, Lehigh University, PA. Research topic: Cell aggregation in the blood of snails.
9/77-8/79	Graduate student in cell biology Laboratory of Dr. Robert Schlegel, Pennsylvania State, University, PA. Research topic: Purification of RNA polymerase I, rRNA synthesis and SV40 T antigen
9/79-2/85	Graduate student in biochemistry and molecular biology Laboratory of Dr. Joel Huberman, Roswell Park Memorial Institute, Buffalo, N.Y. Research topic: Characterization of nuclear matrices from *Saccharomyces cerevisiae*
3/85-2/87	Postdoctoral fellow in molecular biology, yeast genetics Laboratory of Dr. David Beach, Cold Spring Harbor, Laboratory, N.Y. Research topic: Characterization of the cell cycle gene *cdc2* in *Schizosaccharomyces pombe*
3/87-12/89	Postdoctoral fellow in molecular biology, yeast genetics Laboratory of Dr. David Frendewey, Cold Spring Harbor, Laboratory, N.Y. Research topic: Identification and characterization of pre-mRNA splicing mutants in *Schizosaccharomyces pombe*

Academic appointments

1/90-6/96	Assistant Professor, Pharmacology and Molecular Biology Department University of Health Sciences/The Chicago Medical School, IL. Research topic: Cloning and characterization of the splicing factor *prp2* in *Schizosaccharomyces pombe*
7/96-6/03	Associate Professor, Cellular and Molecular Pharmacology Department The Chicago Medical School and School of Graduate and Postdoctoral Studies Rosalind Franklin U. of Medicine and Science Research topic: Identification of fission yeast pre-mRNA splicing factors, characterizing the similarities between the fission yeast and human spliceosome,
7/03-6/06	Associate Professor with tenure, Cellular and Molecular Pharmacology Department The Chicago Medical School and School of Graduate and Postdoctoral Studies Rosalind Franklin U. of Medicine and Science Research topic: identification of splicing factors that regulate of FosB splicing after chronic cocaine administration
7/06-6/08	Associate Professor with tenure and Vice Chair Cellular and Molecular Pharmacology Department The Chicago Medical School The School of Graduate and Postdoctoral Studies Rosalind Franklin U. of Medicine and Science Research topic: Identification of splice variants as molecular markers in a chronic MPTP mouse model of Parkinson's disease
7/08-8/13	Associate Professor with tenure, Cellular and Molecular Pharmacology Department The Chicago Medical School and School of Graduate and Postdoctoral Studies Rosalind Franklin U. of Medicine and Science Research topic: identification of splice variant biosignatures for Parkinson's disease and atypical Parkinsonian disorders in human blood
9/13-present	Professor with tenure, Cellular and Molecular Pharmacology Department The Chicago Medical School and School of Graduate and Postdoctoral Studies Rosalind Franklin U. of Medicine and Science Research topic: identification of biomarkers for Parkinson's disease and atypical Parkinsonian disorders in human blood

Research Interests

Identification of pre-symptomatic splice variant biomarkers for Parkinson's Disease in blood in at risk individuals.

Identification of progression markers for Parkinson's Disease in blood

Identification of Progressive Supranuclear Palsy risk markers in human blood.

Identification of molecular networks that are dysregulated in the early stages of Parkinson's disease.

Investigating the potential of the transcription factor HNF4alpha as a potential therapeutic target of Parkinson's disease.

Certifications

2008- present	IRB approval for Human Studies
2008-present	Certified Group Leased for Respiratory Health Association of Metropolitan Chicago's Courage to Quit Program 2/22/08
2006	Human Participants Protection Education for Research Teams 3/31/06
2006	Working with the IACUC Research Training 3/28/06
2006	Working with Rats in Research Settings Research Training 3/28/06
2006	Working with Mice in Research Settings Research Training 3/28/06

Fellowships

Cold Spring Harbor Fellowship, 1989
Award from Cold Spring Harbor Institutional Research Grant funded by the American Cancer Society, 1988-1989
Nucleic Acid Group of Buffalo Fellowship, 1983-1984.
University of Buffalo Fellowship, 1979-1983, 1984-1985.
Pennsylvania State University Teaching Assistantship, 1977-1979.

Awards and Honors

Presidential Service Award RFUMS, 2012
EMBO Travel Award, RNA disease conference, Rome, Italy, 2008
Sigma Xi Research Award, 1985
Phi Kappa Phi, 1979
Phi Beta Kappa, 1977

Memberships and Affiliations

Society for Neuroscience
RNA Society
The International Fission Yeast Society
American Association for the Advancement of Science
American Society of Biochemistry & Molecular Biology
The International Community of Yeast Genetics and Molecular Biology
The Genetics Society of America
Sigma Xi Honor Society
Midwest Yeast Society

TEACHING

Classroom Teaching

Chicago Medical School

1996-present	MCMP 600 Foundations in Medical Pharmacology. Cancer drugs, gene therapy, immunopharmacology, pharmacogenomics, anti-viral medications and alternative medications. (8-12 h/yr., 180 M2 students)
1996-present	MCMP 600 Medical Pharmacology Small Group Problem Solving/Patient Oriented Problem Solving. **Course Director 2006-present)**

medications to treat hypertension, cancer, Parkinson's disease, asthma, diabetes and psychopharmacology
(11 h/yr., 20-25 M2 students)

2011-2012 MBCH508 Clincial Genetics
Session 1: Autosomal dominant inheritance-familial adenomatous polyposis
Session 2: Autosomal dominant inheritance- breast cancer
Session 3: Autosomal recessive inheritance- cystic fibrosis.
Session 4: X-linked dominant inheritance- fragile X
Session 5: Autosomal disorder- Down's syndrome
Session 6: Heteroplastic Mitochondrial Inheritance- myoclonic epilepsy with ragged-red fibers
(18 h/yr., 11 M1 students and Pharmacy3)

2014-present MMTD509-14SP-YPHS509-14SP Epidemiology
(4 h/yr., 5-6 M1 students)

School of Graduate and Postdoctoral Studies

1990, 1992 Advanced Topics in Eukaryotic Gene Expression, **Course Director**
DNA replication, pre-mRNA splicing, RNA biology

1991-2005 MTD714, BC5238, CMP5622, MV5562, Advanced Molecular Biology, **Course Director (1991-2003)**
DNA replication, pre-mRNA splicing, RNA biology, telomerase, cell cycle
(12 h/yr., 5-20 G1 & G2 students)

1991-1995 Molecular and Cellular Sciences Journal Club, **Course Director**
(8 h/yr., 10-20 G1-G5 students)
(9 h/yr., 2-10 G1 students)

1992 Molecular and Cellular Biology of Hormone Action
(4 h/yr., 2-10 G2 & G3 students)

1994 Ethics in Biomedical Research, **Course Director**
(4 h/yr., 10-20 G1-G5 students)

1999-2003 MTD745 Molecular and Genetic Basis of Disease, **Course Director**
Splicing defects in thalassemias, hemophilia, Tay-Sachs, cystic fibrosis, retinoblastoma, trinucleotide repeat disease, RNA editing, gene therapy
(8 h/yr., 80- 90 Basic science students)

2006 MTD710 Brain Frontiers: Advanced Topics in Neuroscience Research
Regulating pre-mRNA splicing in the CNS
(2 h/yr., 2-10 G2 & G3 students)

2007-2012 GIGP502 MCBII, **Course co-Director (2009-2012)**
miRNAs, splicing and RNA binding proteins
(9 h/yr., 2-10 G1 students)

2008-2012 GCMP700 Teaching in Pharmacology, **Course Director**
(2-5 G2, G3, G4 & G5 students)

2009 Ethics and Compliance in Biomedical Research
(2 h/yr., 2-10 G2 students)

2010 GCMP608 Research skills: Poster presentations and grant writing
(4 h/yr., 2-10 G2 & G3 students)

2011-present GCMP 601 and GCMP 602: Neuropharmacology
Pharmacogenomics of neurodegenerative disorders
(2 h/yr., 2-10 G2 students)

Per need GCMP 545 Advanced Molecular Pharmacology, **Course Director**

School of Related Health Sciences

1997-2003 HPAS538 Physicians Assistants Medical Pharmacology, (cancer drugs, gene therapy, immunopharmacology and anti-viral medications)

1999-2000	Physical Therapy Pharmacology, (cancer drugs) (2 h/yr., 50-60 Physicians Therapy students)
2004-2006	PBBS 601 Pharmacology (cancer drugs, gene therapy, immunopharmacology) (4 h/yr., 50-60 Podiatry and Physicians Assistants students)
2010-2012	PBBS 601 Pharmacology (antiviral drugs) (2 h/yr., 50-60 Podiatry and Physicians Assistants students)

Other Teaching Contributions within RFUMS

2002-2006	CPR review course 2002, CMS

Other Teaching Contributions Outside of RFUMS

1977-1979	Microbiology lab, Pennsylvania State University (1 semester/yr, 20-30 undergraduate students)
1993	Cold Spring Harbor Laboratory in the Molecular Genetics, Cell Biology & Cell Cycle of Fission Yeast (1 day, 20-30 post-graduate students)

Student Development

Research Assistant Professors/Postdoctoral Fellows Trained

Svetlana Ehmann	MS, Research Associate
Jinyuan Li	PhD, Research Assistant
Patricia Loomis	PhD, Research Assistant Professor
Thomas Jeitner	PhD, Research Assistant Professor
Jose Santiago	MS, Research Assistant

Scientists Hosted for Sabbatical

Guru Rao	Scientist, Pioneer Hi-Bred
Isabell Witt	Assistant Professor, Max-Plank Institute, Berlin

Graduate Student Thesis Committees within RFUMS/Finch U.

Kelly Wentz-Hunter	Pharmacology and Molecular Biology **Thesis advisor**, Ph.D. 1997 Assistant Professor Roosevelt University
Fikret Sahin	Microbiology and Immunology **Thesis co-advisor**, Ph.D 2001
Joe Litwak	Pharmacology and Molecular Biology
Ji Zhang	Pharmacology and Molecular Biology
Xuan Liu	Microbiology and Immunology
Steve Aller	Microbiology and Immunology
Geraldine New	Cell Biology and Anatomy
Missok Lee	Biological Chemistry
Pat Halloran	Microbiology and Immunology
Hong-gang Shen	Biological Chemistry
Bingyi Yao	Biological Chemistry
Karuna Naik	Pharmacology and Molecular Biology
Susan Sweeney	Microbiology and Immunology
Jie Zhang	Microbiology and Immunology
Joel Saban	Pharmacology and Molecular Biology
Yongjun Tan	Cellular and Molecular Pharmacology
Rob Buechler	Cellular and Molecular Pharmacology
Leyla Akman	Microbiology and Immunology

Barbara Sum	Microbiology and Immunology
Chanwit Tribuddharat	Microbiology and Immunology
Lisa Monteggia	Neuroscience
Yng Chen	Biochemistry and Molecular Biology
Steven Chao	Neuroscience
Jie Hyun Bae	Microbiology and Immunology
Mark Fons	Cellular and Molecular Pharmacology
Todd Swanson	Microbiology and Immunology
Sean Parks	Microbiology and Immunology
Fritz Jean-Pierre Jr.	M.D. with Distinction in Research Program
Michael Hinds	Biochemistry and Molecular Biology
Jian Qin	Microbiology and Immunology, SUNY Downstate
Yoon-Sang Kim	Microbiology and Immunology
Victor Marinescu	**Thesis advisor**, Cellular and Molecular Pharmacology, PhD 2006
Kelly Conrad	Neuroscience
Amy Boudreau	Neuroscience
Kayoko Waki	Microbiology and Immunology
Sujoy Dutta	Microbiology and Immunology
Yamin Wang	Biochemistry and Molecular Biology
Sunaina Yadav	Biochemistry and Molecular Biology
Jeffrey Huang	Neuroscience, **Chair**
WaiChong (Vivian) Wong	Cellular and Molecular Pharmacology
Mallory Havens	Cell Biology and Anatomy

Graduate Student Thesis Committees outside of RFUMS

Durkeshwari Anbalagan	Physiology, Yong Loo Lin School of Medicine, National U. of Singapore

MD with Distinction Thesis Committees

Justin Wikle	**Thesis advisor**, Cellular and Molecular Pharmacology
Clarabel Wee	Cell Biology and Anatomy
William Pearse	Cellular and Molecular Pharmacology

High School and Undergraduates Trained

Jeremy Reimers	Carthage College, 2002
Nicole Runkle	Illinois Math and Science Academy, 2010
Svetlana Portman	Tutor High School Biology
Stacey Seidl	DePaul University, 2011-2012
Noah Van Handel	Washington Montessori School, Litchfield, CT, 2014

SERVICE

Committee Service for RFUMS or Chicago Medical School

1990	Molecular and Cellular Sciences Selection Committee
1992-present	Molecular & Cellular Sciences Seminar Committee, **Chair** 1994-1996
1993-1995	Senator-at-Large, district IV
1996-2003	Library Committee
1996-2003	Recombinant DNA Advisory Committee
1998-2002	Cellular and Molecular Pharmacology Senator
1998-2003	Education and Planning Committee
1998-2004	Faculty-Student Forum Committee
1999-2003	Institutional Biosafety Committee
2001-2003	Rules Committee, **Chair**, member 1998-2002
2002	Judge of Summer Research Fellow Poster Session
2002-2004	Senator-at-Large District IV

2002-2008 Faculty Awards Committee, **Chair** 2004-2008
2004-2006 Institutional Biosafety Committee
2004-2008 Vertical Integration Group: General Principles/Introductory
2004-2008 Senator, district II
2005-2006 Graduate Student Admissions Committee
2005-2006 Research Review Panel Graduate Student Admissions Committees
2005-2010 Faculty Appointment, Promotions and Tenure Committee
2005-2009 RNA quantification laboratory, **Director**
2005-2009 Research Core Facilities Oversight Committee
2005-2010 Faculty Appointment, Promotions and Tenure Committee
2005-2012 SEPAC Appeals Board, Chair
2008-2010 Senator at large

2010-2011 Educational Affairs Committee
2010-2011 Examination subcommittee of the Educational Affairs Committee
2011-2012 Strategic Planning Committee
2011-2012 SP TEAM Subgroup B – Stakeholder involvement and implementation
2011-2012 SSG - Prepare Presentation for University Community
2009-present Y1/Y2 subcommittee of the Educational Affairs Committee
2010-present CMP Senator, district II
2013-present Senate Executive Council, CMS councilor
2013-present CMS Admissions Committee
2013-present FAPT Committee, Dean's representative

Committee Service for Cellular and Molecular Pharmacology Department

1990-1992 Pharmacology and Molecular Biology Seminar Committee, **Chair**
1990-1995 Pharmacology and Molecular Biology Graduate Student Recruitment Committee, **Chair**
1991-1992 PMB Graduate Student Advisory Committee
1991-1992 PMB Course Review/Evaluation Committee
1992-1993 Committee for Development & Evaluation of Molecular Biology Oriented Courses
1997-1998 PMB/CMP Journal Club Committee
1997-1999 Graduate Student Evaluation Committee
1997-1999 Graduate Student Recruitment Committee
1997-2004 CMP Course Evaluation Committee
1997-2007 CMP Faculty Recruitment Committee
1998-1999 CMP Graduate Student Advisory Committee
2004 Peer Review Assessment Committee
2004-2012 Faculty Appointment, Promotions and Tenure Committee**, Chair 2004-2007**, **2009-2012**
2005 CMP Faculty Evaluation Committee for Annual Reports
2005-2007 CMP Seminar Organizer
2006 Work Load Model Committee
2006 Chair Evaluation Committee
2006-2008 Vice Chair
2008-present Space committee, chair
2010-present Graduate Student Oversight Committee
2013-present Chair of Seminar Committee

Other Service

1983 Graduate Student Selection Committee, Buffalo, NY
2009-2012 Career mentoring junior faculty, postdoctoral fellows and graduate students
Mirek Dundr, Michelle Hastings, Dom Duelli, Virginie Bottero, Bob Marr

Service for the Scientific Community

Review of Grant Proposals

1991	Biomedical Research Support Grant reviewer at CMS
1997-1998	Ad hoc reviewer for National Institute on Aging; National Institute on Neurological Disorders and Stroke, National Institutes of Health
2000-2002	National Science Foundation
2004-2006	Philip Morris External Research Program
2007	Pennsylvania Interim Review 2nd Panel
2008	Pennsylvania Performance Review, 07-08 cycle A
2013	Tucino Animal Protocol Review for the Neurocenter of Southern Switzerland
2013	DePaul-RFUMS Pilot Grant Review Committee

Review of Manuscripts for Journals and Books

Current Genetics
Current Cancer Drug Targets
Elsevier: The Basal Ganglion
EMBO Molecular Medicine
FEBS Journal
Frontiers in Neuropharmacology, Editorial Board
Genetics
Genomics and Proteomics
Int. J. Environ. Res. Public Health
Journal of Clinical & Experimental Pharmacology, Editorial Board
Journal of Biological Chemistry
Journal of Molecular Biology
Journal of Neurochemistry
Journal of Neuropharmacology
Journal of Neuroscience Research
Molecular and Cellular Biology
Neuron
Nucleic Acid Research
PLoS ONE
Protein and Cell
RNA
Toxicology Sciences
Yeast

Meeting Organizer

1983	Nuclear Matrix Mini Symposium Committee, Buffalo, NY
1983	Nucleic Acid Group of Buffalo Organizational Committee, Buffalo, NY
2002	Organizer of the Genes to Protein Symposium of the International Fission Yeast Meeting in Kyoto

Scientific Advisor

1989	The conference for selecting the gene names for splicing mutants, CSHL, NY
1999-2003	Sanger Centre for the RNA processing factors of the fission yeast database
2000	Proteome, Inc. on the PombePD
2007-present	AbaStar MDx
2009-present	Science Advisory Board, Bioinformatics, LLC
2011	PLoS One efocus group
2012	Healthcare Consulting Datamonitor Group

Community

2009-present	Certified by Respiratory Health Association as Group Leader for Chicago's Courage to Quit
2009	Judge for postdoctoral fellow posters at Society for Neuroscience Chicago Chapter

Professional Societies

Society for Neuroscience
RNA Society
Midwest RNA Society
The International Fission Yeast Society
American Association for the Advancement of Science
American Society of Biochemistry & Molecular Biology
The International Community of Yeast Genetics and Molecular Biology
The Genetics Society of America
Sigma Xi Honor Society
Midwest Yeast Society

RESEARCH AND SCHOLARSHIP

Grants

Active

Department of Defense USAMRAA, W81XWH-13-1-0025, Splice Variant Biomarkers for Parkinsons Disease, 3 years, PI, 4/15/13-41/14/16, $1,032,778 total costs.

The Michael J. Fox Foundation for Parkinson's Research, Whole Blood RNA Biomarkers of Parkinson's Disease, 6 months, PI, 3/4/2014-8/3/2014, $55,119 total costs.

Pending

None

Completed

1985-1987	National Institute of General Medical Sciences, 1F32 GM010831, PI, 100% effort Characterization of the cdc2 gene product in fission yeast, $75,000 direct costs
1990-1992	American Cancer Society, Illinois Division, #90-37, PI, 50% effort Molecular Characterization of Pre-mRNA Splicing Genes, $57,290 direct costs
1992	Biomedical Research Support Grant Award, BRSG 2-556-855, PI Cloning Human Pre-mRNA Splicing Factors, $4,800 direct costs
1992-2000	National Institute of General Medical Sciences, 5R01 GM47487, PI, 25% effort Fission yeast pre-mRNA splicing factors, $689,247 direct costs
1995-1997	American Cancer Society Junior Faculty Research Award, JFRA-545, PI, 50% effort Characterization of the splicing factor PRP2, $90,500 direct costs
2000-2002	National Institute of Drug Abuse, R03 DA136703, PI, 25% effort Cocaine regulation of FosB splicing, $100,000 direct costs
2002-2005	National Institute of Drug Abuse, R01 DA15367, PI, 25% effort Cocaine regulation of FosB splicing, $450,000 direct costs
2005-2008	Department of Defense USAMRAA NETRP program, W81XWH-05-1-0580, co-I, 25% effort Identification of splice variants as molecular markers in Parkinson's disease, $517,649 direct costs +$110,075 supplement for human studies
2009-2013	Department of Defense USAMRAA, W81XXWH-09-1-0708, PI, 25% effort Identification of Splice Variants as a Biosignature for Parkinson's Disease, $479,010 direct costs
2012-2013	CurePSP Foundation Award, #507-13, PI, 5% effort Splice variant risk markers for progressive supranuclear palsy, $75,000 direct costs

Publications

Peer-reviewed journal articles

1. Potashkin, J.A. and Schlegel, R.A. A possible mechanism by which SV40 T-antigen stimulates r,RNA synthesis. Cell Bio. International Reports 4: 399- 406 (1980).

2. Potashkin, J.A., Zeigel, R.F. and Huberman, J.A. Isolation and initial characterization of residual nuclear structures from yeast. Exp. Cell Res. 153: 374-388 (1984).

3. Potashkin, J.A. and Huberman, J.A. Characterization of DNA sequences associated with residual nuclei of *Saccharomyces cerevisiae*. Exp. Cell Res. 165: 29- 40 (1986).

4. Draetta, G, Brizuela, L., **Potashkin, J.,** and Beach, D. Identification of p34 and p13, human homologs of the cell cycle regulators of fission yeast encoded by *cdc2+* and *suc1+*. Cell 50:319-325 (1987).

5. Potashkin, J.A. and Beach, D.H. Multiple phosphorylated forms of the product of the fission yeast cell division cycle gene *cdc2+*. Current Genetics 14:235-240 (1988).

6. Potashkin, J., Li, R., Frendewey, D. Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. EMBO J 8:551-559 (1989), PMC400840.

7. Potashkin, J. and Frendewey, D. Splicing of the U6 RNA precursor is impaired in fission yeast pre-mRNA splicing mutants. Nucl. Acids Res. 17:7821-7831 (1989), PMC334889.

8. Frendewey, D., Barta, I., Gillespie, M. and **Potashkin, J.** *Schizosaccharomyces* U6 genes have a sequence within their introns that matches the B box consensus of tRNA internal promoters. Nucl. Acids Res. 18:2025-2032 (1990), PMC330678.

9. Potashkin, J. and Frendewey, D. A mutation in a single gene of *Schizosaccharomyces pombe* affects the expression of several snRNAs and causes defects in RNA processing. EMBO J. 9:525-534 (1990), PMC551696.

10. Potashkin, J. A., Derby, R. J. and Spector, D. L. Differential distribution of factors involved in pre-mRNA processing in the yeast cell nucleus. Mol. Cell. Biol. 10:3524-3534 (1990), PMC360787.

11. Potashkin, J., Naik, K. and Wentz-Hunter, K. U2AF homolog required for splicing *in vivo*. Science 262:573-575 (1993), PMID: 8211184. Cited as a focus topic in This Week in Science Science 262:485 (1993).

12. Wentz-Hunter, K. and **Potashkin, J**. The evolutionary conservation of the splicing apparatus between fission yeast and man. Nucleic Acids Symposium Series 33:226-228 (1995).

13. Wentz-Hunter, K. and **Potashkin, J.** The small subunit of the splicing factor U2AF is conserved in fission yeast. Nucl. Acids Res. 24:1849-1854 (1996), PMC145878.

14. Potashkin, J., Wentz-Hunter, K. and Callaci, J. BTF3 is evolutionarily conserved in fission yeast. Biochim. Biophys. Acta 1308:182-184 (1996).

15. McKinney, R., Wentz-Hunter, K, Schmidt, H. and **Potashkin, J.** Molecular characterization of a novel fission yeast gene spUAP2 that interacts with the splicing factor spU2AF59. Current Genetics 32:323-330 (1997).

16. Gozani, O., **Potashkin, J.** and Reed, R. Recruitment of U2 snRNP to the branchpoint sequence via direct interactions with U2AF, Mol. Cell. Biol 18:4752-4760 (1998), PMC109061.

17. Potashkin, J., Kim, D., Fons, M., Cannon, B., Humphrey, T. and Frendewey, D. Cell division cycle defects associated with fission yeast pre-mRNA splicing mutants, Current Genetics 34: 153-163 (1998).

18. Beales, M., Flay, N., McKinney, R., Habara, Y., Ohshima, Y. Tani, T., and **Potashkin, J.** Mutations in the large subunit of U2AF disrupt pre-mRNA splicing, cell cycle progression and nuclear structure. Yeast 16:1001-1013 (2000).

19. Käufer, N. F. and **Potashkin, J.** Analysis of the splicing machinery in fission yeast: a comparison with budding yeast and mammals. Nucl. Acids Res. 28:3003-3010 (2000), PMC108416.

20. Ochotorena, I.L., Hirata, D., Kominami, K., **Potashkin, J.,** Sahin, F., Wentz-Hunter, K., Gould, K. L., Sato, K. Yoshida, Y., Vardy, L.. and Toda, T. Conserved Wat1/Pop3 WD-repeat protein of fission yeast secures genome stability through microtubule integrity and may be involved in mRNA maturation. J. Cell Science 114:2911-2920 (2001).

21. Wood, V., Gwilliam, R., Rajandream M-A., Lyne, M., Lyne, R., Stewart, A., Sgouros, J.,Peat, N., Hayles, J Baker, S., Basham, D., Bowman, S. Brooks, K., Brown, D,. Brown, S., Chillingworth, T., Churcher, C. , Collins, M., Connor R., Cronin, A., Davis, P., Feltwell, T., Fraser A., Gentles, S., Goble, A., Hamlin, N., Harris, D., Hidalgo, J., Hodgson, G., Holroyd, S., Hornsby, T., Howarth, S., Huckle, E. J., Hunt, S., Jagels, K., James, K., Jones, L., Jones, M., Leather, S., McDonald, S., McLean, J., Moule S., Mungall, K., Murphy, L., Niblett, D., Odell, C., Oliver, K., O'Neil, S., Pearson, D., Quail, M. A., Rabbinowitsch, E., Rutherford, K., Rutter, S., Saunders, D., Seeger, K., Sharp, S., Skelton, J., Simmonds, M., Squares, R., Squares, S., Stevens, K.,Taylor, K., Taylor, R. G., Walsh, S., Warren, T., Whitehead, S., Woodward J., Volckaert, G., Aert, R., Robben, J., Grymonprez, B., Weltjens, I., Vanstreels, E., Rieger|, M., Schäfer| M., Müller-Auer| S.,Gabel| C., Fuchs|, M., Fritzc, C., Holzer, E., Moestl D., Hilbert, H., Borzym, K., Langer, I.,Beck, A., Lehrach, H., Reinhardt R., Pohl, T. M., Eger, P., Zimmermann, W., Wedler, H., Wambutt, R., Purnelle, B., Goffeau, A., Cadieu, E., Dréano, S., Gloux, S., Lelaure, V., Mottier, S., Galibert, F., Aves|, S. J., Xiang|, Z., Hunt| C., Moore|, K., Hurst, S. M. |, Lucas, M., Rochet, M., Gaillardin, C., Tallada, V. A., Garzon, A., Thode, G., Daga, R. R., Cruzado, L., Jimenez, J., Sánchez, M., del Rey, F., Domínguez, A., Revuelta J. L., Moreno, S., Armstrong, J., Forsburg, S., Cerrutti, L., Lowe, T., McCombie, W. R., Paulsen, I., **Potashkin**, **J.**, Shpakovski, G., Ussery, D., Barrell, B. G., Nurse, P. The Genome Sequence of the Eukaryote Fission Yeast *Schizosaccharomyces pombe*. Nature 415:871-880 (2002), PMID: 11859360.

Cited in News and Views: Nature 415:845 (2002),

Wood, V. et. al. Corregenda The Genome Sequence of the Eukaryote Fission Yeast *Schizosaccharomyces pombe*. Nature 421:94 (2003).

22. Potashkin, J. and Meredith, G. The Role of Oxidative Stress in the Dysregulation of Gene Expression and Protein Metabolism in Neurodegenerative Disease, Antioxidant and Redox Signaling 8:144-151 (2006), PMID: 16487048.

23. Alibhai, I.N., Green, T.A., **Potashkin, J.A**., Nestler, E.J. Regulation of fosB and DfosB mRNA Expression: In Vivo and In Vitro Studies. Mol. Brain Res. 1143:22-33 (2007), PMC1880876, NIHMSID21200.

24. Li, X., Xi, X., Zhou, L., Catera, D., Rote, N., **Potashkin, J.,** Abdul-Karim, F. and Gorodeski, G.I. Decreased Expression of P2X7 in Endometrial Epithelial Pre-Cancerous and Cancer Cells. Gynecol. Oncol. 106:233-43 (2007), PMC2398694.

25. Marinescu, V., Loomis, P., Ehmann, S., Beales, M. and **Potashkin, J**. Regulation of Retention of FosB Intron 4 by PTB, PLoS One 2(9): e828 (2007), PMC1952174.

26. Potashkin, J. A, Kang, U.J. Loomis, P. A. Jodelka, F. D., Ding, Y. and Meredith, G. E. MPTP administration in mice changes the ratio of splice isoforms of fosB and rgs9, Brain Res. 1182:1-10 (2007) PMID: 17936734.

27. Meredith, G.E, Totterdell, S, **Potashkin, J.A**, Surmeier, D. S. Modeling PD pathogenesis in mice: Advantages of a chronic MPTP protocol. Parkinsonism Related Disorders, 14:S112-S115 (2008), PMC2547123, NIHMSID63899.

28. Zhou,' L., Qi, X., **Potashkin, J.A**, Luo, L. Fu,W., Abdul-Karim, F.,D., and Gorodeski, G.I. Micro-RNAs miR-186 and miR-150 downregulate expression of the pro-apoptotic purinergic P2X7 receptor by activation of instability sites at the 3'-untranslated region of the gene that decrease steady-state levels of the transcript., J Biol Chem 283:28274-86 (2008), PMC2568908.

29. Potashkin, J.A., Blume, S.R. and Runkle, N.K. Limitations of Animal Models of Parkinson's Disease, Parkinson's Disease, 2011: 658083 (2011). PMID:21209719, PMC658083.

30. Wentz-Hunter, K. and **Potashkin, J.A.** The Role of miRNAs as Key Regulators in the Neoplastic Microenvironment, Molecular Biology International, (2011) Article ID 839872. NIHMSID #309738 PMID:22091413

31. Seidl, S.E. and **Potashkin, J.A.** The Promise of Neuroprotective Agents in Parkinson's Disease, Frontiers in Neuropharmacology, doi: 10.3389/fneur.2011.00068, (2011). PMID:22125548, PMC3221408
Seidl, S.E. and **Potashkin, J.A.** Erratum: The Promise of Neuroprotective Agents in Parkinson's Disease, Frontiers in Neuropharmacology **7**:69.doi: 10.3389/fnins. 2013.00069 (2013)
Top performing article in Frontiers: as of August 2013 3,224 views, 1,198 downloads.

32. Potashkin, J.A., Santiago, J.A., Ravina, B.M., Watts, A. and Leontovich, A.A. Biosignatures for Parkinson's Disease and Atypical Parkinsonian Disorders, PLoS One, 7:1-13, e43595 (2012).
Focus of neurotalk blog: http://neurotalk.psychcentral.com/thread178114.html

33. Santiago, J.A and **Potashkin, J.A**. Shared dysregulated pathways lead to Parkinson's disease and diabetes, Trends in Mol Med, 19: 176-186 (2013).

34. Santiago, J. A., Scherzer, C. R., Harvard Biomarker Study Group, and **Potashkin, J.A**. Specific splice variants are associated with Parkinson's disease, Movement Disorders, 28:1724-7. (2013) doi:10.1002/mds.25635. Epub 2013 Sep 20. PMID: 24108702

35. Santiago, J.A and **Potashkin, J.A**. A Network Approach to Diagnostic Biomarkers in Progressive Supranuclear Palsy, Movement Disorders, 29(4):550-5. (2013) doi: 10.1002/mds.2576. PMID:24347522

36. Santiago, J.A and **Potashkin, J.A**. Integrative network analysis unveils convergent molecular pathways in Parkinson's disease and diabetes, PLoS One, 8(12):e83940. (2013) doi: 10.1371/journal.pone.0083940.

37. Seidl, S.E., Santiago, J. A., Bilyk, H. and **Potashkin, J.A.** The Emerging Role of Nutrition in Parkinson's disease**,** Front. Aging Neurosci. (2014) 6:36. PMID: 24639650

38. Santiago, J.A and **Potashkin, J.A**. System-based approaches to decode the molecular links in Parkinson's disease and diabetes, Neurobiology Disease. (2014), in press. PMID:24718034 doi: 10.1016/j.nbd.2014.03.019.

Santiago, J.A and **Potashkin, J.A**. Network analysis identifies HNF4A and SOD2 mRNAs as biomarkers for Parkinson's Disease" to Neurobiology of Aging, submitted.

Book Chapters

1. Potashkin, J.A. and Huberman, J.A. Are specific DNA sequences associated with residual nuclei? in Yeast Cell Biology (J. Hicks ed.) 367-376, Alan R. Liss, New York (1986).

2. Brizuela, L., Draetta, G, **Potashkin, J.,** and Beach, D. Physical association between products of cdc2-positive and sucl-positive genes of fission yeast and between their homologs in mammalian cells. In Nuclear Oncogenes (eds. Alt, F. W. Harlow, E. Ziff, E. B) Cold Spring Harbor, NY: Cold Spring Harbor Lab: 38-42 (1987).

3. Mayes, A. E., **Potashkin, J**. and Beggs, J. Splicing of pre-mRNA introns. In The Frontiers in Molecular Biology Series: The Yeast Nucleus, Eds. P. Fantes and J. D. Beggs, IRL Press, Oxford (2000).

4. Wu, J.Y. and **Potashkin, J.A.**, Alternative splicing in the nervous system. Encyclopedia of Neuroscience, (L.R. Squire, Editor). Oxford: Academic Press, 1:245-251 (2009).

Editorials

1. Potashkin, J.A., Biomarkers Of Neurodegeneration That Would Please A Vampire. Frontiers in Neuroscience. 4:134-135 (2010). NIHMSID # 309729

2. Potashkin, J.A., MiRNAs, Cause or Cure Frontiers in Neuroscience. Research Highlights 4:140 (2010).

3. Santiago, J.A and **Potashkin, J.A**. Network Analysis Accelerates Understanding of Disease Mechanisms. Clin Exp Pharmacol 2013, 3:4, http://dx.doi.org/10.4172/2161-1459.1000e123.

Patents

Screening, Diagnosing, Treating, and Prognosis of Pathophysiologic Status By RNA Regulation (#UHOSP-16328, U.S. patent application Ser. No. 12/450,124 filed 9/11/09).

Splice Variant Specific Messenger RNA Transcripts as Biomarkers of Parkinson's Disease, (#20070087376, U.S. patent application Ser. No. 13/240,821 filed 9/22/11).

Published Database Contributions

GenBank accession number L22577, spU2AF^{59} (1993)
GenBank accession number U48234, spU2AF^{23} (1996)
GenBank accession number U29488, spBTF3 (1996)
GenBank accession number U97681, spUAP2 (1997)
GenBank accession number AF073779, spBBP/SF1 (1998)

Published Abstracts

1. Potashkin, J.A. and Huberman, J.A. Characterization of DNA sequences associated with residual nuclei of *Saccharomyces cerevisiae*, Replication Meeting, Cold Spring Harbor 1985

2. Potashkin, J.A. and Beach, D.H. Characterization of the fission yeast *cdc2* geme, Cell Cycle Meeting, Cold Spring Harbor 1986

3. Potashkin, J.A. and Beach, D.H. Characterization of the fission yeast *cdc2* gene, Interantional Yeast Meeting Edinburgh U.K., 1986

4. Potashkin, J.A. and Beach, D.H. Multiple phosphorylated forms of the product of the fission yeast cell division cycle gene *cdc2*, Cell Cycle Meeting, Cold Spring Harbor 1987

5. Potashkin, J.A. and Beach, D.H. Characterization of the fission yeast CDC2 protein, International Yeast Meeting, Banff, Canada,1987

6. Potashkin, J., Li, R., Frendewey, D. Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. RNA Meeting, Cold Spring Harbor 1988

7. Potashkin, J., Li, R., Frendewey, D. Isolation of a mutant defective in U2 RNA synthesis form Schizosaccharomyces pombe, RNA Meeting, Cold Spring Harbor 1988

8. Potashkin, J., Li, R., Frendewey, D. Characterization of pre-mRNA splicing mutants of fission yeast. International Yeast Meeting, Helsinki, Finland 1989.

9. Potashkin, J., Li, R., Frendewey, D., A *Schizosaccharomyces pombe ts*$^{-}$ mutant with reduced quantities of snRNAs, RNA Meeting, Cold Spring Harbor 1989

10. Potashkin, J., Li, R., Frendewey, D.Unspliced U6 RNA acccumulates in fission yeast pre-mRNA splicing mutants, RNA Meeting, Cold Spring Harbor 1989

11. Potashkin, J. A., Derby, R. J. and Spector, D. L. Differential distribution of factors involved in pre-mRNA processing in the yeast cell nucleus, RNA Meeting, Cold Spring Harbor 1990

12. Frendewey, D., Barta, I., Gillespie, M. and **Potashkin, J.** *Schizosaccharomyces* U6 genes have a sequence within their introns that matches the B box consensus of tRNA internal promoters, RNA Meeting, Cold Spring Harbor 1990

13. F. Lindh and **Potashkin, J.** Cloning fission yeast pre-mRNA splicing genes, RNA Meeting, Cold Spring Harbor 1991

14. F. Lindh and **Potashkin, J.** A novel pre-mRNA splicing factor, RNA Meeting, Keystone Colorado 1992

15. Potashkin, J. Characterization of a pre-mRNA splicing factor that is homologous to mammalian $U2AF^{65}$, Madison, WI , International Yeast Meeting, 1993.

16. Potashkin, J. A novel pre-mRNA splicing factor that is homologous to mammalian $U2AF^{65}$, RNA Meeting, Cold Spring Harbor 1993

17. Naik, K and **Potashkin, J.** Genetic analysis of *prp2*$^{-}$ mutants, RNA Meeting, Madison, WI , 1994

18. Wentz-Hunter, K. and **Potashkin, J**. Functional complementation of a fission yeast pre-mRNA processing mutant by the human splicing factor $U2AF^{65}$, RNA Meeting, Madison, WI , 1994

19. Wentz-Hunter, K., Noskina, Y. and **Potashkin, J**. Characterization of the pre-mRNA splicing factor $yU2AF^{59}$: Identification of interacting proteins and snRNAs , RNA Meeting, Cold Spring Harbor 1995

20. Wentz-Hunter, K., McKinney, R. and **Potashkin, J**.Characterization of the large and small subunits of spU2AF, RNA Meeting, Madison, WI , 1996

21. Potashkin, J, Wentz-Hunter, K., McKinney, R, Witt, I. and Beales, M. Characterization of spU2AF. RNA Meeting, Banff, Canada,1997

22. Gozani, O., **Potashkin, J**., Wang, C**.** and Reed, R. SAP155-U2AF interactions are conserved from *S. pombe* to humans. RNA Meeting, Cold Spring Harbor 1997

23. Potashkin, J, Wentz-Hunter, K., McKinney, R, Witt, I., Schmidt, H. and Beales, M. Characterization of spU2AF, RNA Meeting, Cold Spring Harbor 1997

24. Beales, M., McKinney, R., Flay, N., Habara, Y., Ohshima, Y. Tani, T., and **Potashkin, J.** The large subunit of U2AF plays a role in pre-mRNA splicing, cell cycle progression and nuclear structure. RNA Meeting, Madison, WI , 1998

25. Sahin, F., Beales, M, Käufer, N.F. and **Potashkin, J**, Co-expression of the large and small subunits of spU2AF disrupts some protein interactions and enhances others, RNA Meeting, Cold Spring Harbor 1999

26. Potashkin, J, A key role of the splicing factor spU2AF59 in splicing complex assembly, International Fission Yeast Meeting, Edinburgh, U.K., 1999

27. Käufer, N. F. and **Potashkin, J.** Analysis of the splicing machinery in fission yeast: a comparison with budding yeast and mammals, RNA Meeting, Madison, WI , 2000

28. Potashkin, J., The fission yeast splicing complex., RNA processing meeting, Cold Spring Harbor, 2001.

29. Potashkin, J, An overview of gene expression in fission yeast, International Fission Yeast Meeting, Kyoto, Japan, 2002

30. Potashkin, J . Ehmann, S, Strunin, V., Marinescu, V. and Beales,M. Splicing regulation of the transcription factor FosB, RNA 2003, Vienna, Austria

31. Potashkin, J . Ehmann, S, Strunin, V., Marinescu, V. and Beales,M. Factors that regulate pre-mRNA splicing of fosB. Society for Neuroscience 33rd Annual Meeting, 2003. New Orleans, P897.13

32. Ehmann, S., Marinescu, V., Li, J. Strunin, V. and **Potashkin, J.** PTB Regulates FosB Splicing, RNA2004, Madison, WI

33. Li, J., Hong, W. Reimers, J. Dervan, A., Meredith, G. and **Potashkin, J**. Disruption of Splicing Regulation in Parkinson's Disease, RNA2004, Madison, WI

34. Marinescu, V. Ehmann, S., Li, J., Strunin V. and **Potashkin, J**, Regulation of FosB splicing in cocaine addicted animals, Society for Neuroscience 34th Annual Meeting, 2004, San Diego, CA, P463.11

35. Potashkin, J, Reimers, J., Hong, W. Dervan, A. Meredith, G. and Li, J., Disruption of splicing regulation in Parkinson's disease, Society for Neuroscience 34th Annual Meeting, 2004, San Diego, CA

36. Marinescu, V. Ehmann, S. and **Potashkin, J**, PTB regulation of FosB splicing. Eukaryotic RNA processing 2005, Cold Spring Harbor, NY.

37. Marinescu, V. Ehmann, S. and **Potashkin, J**, PTB interacts with splicing regulatory elements of FosB. Society for Neuroscience 35th Annual Meeting, 2005, Washington DC, P226.4.

38. Alibhai I, Green, T. **Potashkin, J**. and Nestler, E. Modulation of FosB mRNA isoforms. Neuroscience 35th Annual Meeting, 2005, Washington DC., P451.15.

39. Jeitner, T., Meredith, G and **Potashkin, J**, FosB splicing regulation is disrupted in an MPTP model. WPD Congress 2006, Washington DC. Movement Disorders

40. Marinescu, V. and **Potashkin, J**, Dopamine D1 Receptor Stimulation Alters the Distribution of nPTB and Induces ΔFosB Expression, RNA2006, Seattle, WA.

41. Potashkin, J, , Loomis, P., Pitner, J., Leitermann, R. and Meredith, G. FosB Splicing Regulation is Disrupted in the Blood of an MPTP Rodent Model of Parkinson's Disease, RNA2006, Seattle, WA.

42. Loomis, P., Pitner, J., Jodelka,, F. Meredith, G. and **Potashkin**, J. Identification of mRNA splice variants in an acute model of Parkinson's disease. Neuroscience 36th Annual Meeting, 2006, Washington DC

43. Potashkin, J,, Marinescu, V. and Loomis, P. The D1 Receptor Mediates DeltaFosB mRNA Expression in Postnatal Nucleus Accumbens Cultures Keystone Addiction meeting 2007 Santa Fe, NM, P216.

44. Loomis, P., Wikle, J. and **Potashkin, J**,, Regulation Of Fos B Pre-mRNA Splicing. Eukaryotic mRNA processing. 2007. Cold Spring Harbor, N.Y.

45. Potashkin, J,, Loomis, P., Ding, Y., Jodelka, F., Jackolin, J., Kang, U.J. and Meredith, G. Ache Splicing Regulation Is Disrupted In The Brain And Blood Of An MPTP Mouse Model Of Parkinson's Disease. Eukaryotic mRNA processing. 2007. Cold Spring Harbor, N.Y.

46. Zhou, L., **Potashkin, J**., Qi, X., and Gorodeski, G.I. Enhanced instability of $P2X_7$ mRNA in cancer epithelial cells. Eukaryotic mRNA processing. 2007. Cold Spring Harbor, N.Y.

47. Potashkin, J., Loomis, P., Ding, Y. Jodelka, F., Jackolin, J. Kang, UJ and Meredith, G. Dysregulation of AChE splicing in acute and chronic models of Parkinson's disease, Neuroscience 37th Annual Meeting, 2007, San Diego, CA.

48. Potashkin, J NDUFS4 splicing regulation is disrupted in the brian and blood of MPTP mouse models of Parkinson's disease, 2008, Rome, Italy.

49. Zhou, L., Qi, X., Luo, L. Agarwal, M., Skomorovska-Prokvolit, O. Fu, W. **Potashkin, J.** and Gorodeski, G. Poly(ADP-ribose) polymerase (PARP) decreases apoptosis and stimulates growth of HeLa cells by decreasing stability of $P2X_7$ mRNA, 99th AACR Annual Meeting, San Diego, CA. abstract #2692

50. Potashkin, J, Scherzer, C, Ravina, B, Watts, A and Leontovich, L. A Splice Isoform Signature Of Parkinson's Disease In Blood, WPD Congress 2010, Glascow, Scotland Movement Disorders, P01.06.

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52. Wentz-Hunter, K. , **Potashkin, J.** , V. Liakaite, V. Leverenz, A. , Veal, J.. Identification of miRNA Biomarkers of Oxidative Stress, a Risk Factor for Glaucoma, in Bovine Trabecular Meshwork Cells. MicroRNAs and Human Disease**,** Keystone meeting, Banff, Canada 2011.

53. Santiago, J, Leontovich, A, Scherzer, C, Ravina, B, Watts, A and **Potashkin, J.** Progress toward identifying a peripheral blood biosignature of Parkinson's disease, Neuroscience 41th Annual Meeting, 2011, Washington DC. P49.24.

54. Santiago, JA, Hirschy, R., Ravina, BM, Watts, A., Leontovich, A.A, and **Potashkin, JA.,** Splice variant biosignatures of Parkinson's Disease and Atypical Parkinsonian Disorders. Neurodegenerative diseases, Cold Spring Harbor Laboratory 2012.

55. Santiago, JA, Scherzer, C, Harvard Biomarker Study Group, and **Potashkin, JA,** Splice variant specific blood biomarkers of Parkinson's disease, RNA 2013, Davos, Switzerlalnd.

Invited International Seminars

1986 International Fission Yeast Meeting, Edinburgh, Scotland
1987 Imperial Cancer Research Fund, London
1997 RNA '97, The annual meeting of the RNA society, Banff, Canada
1999 International Fission Yeast Meeting, Edinburgh, Scotland
2002 International Fission Yeast Meeting, Kyoto, Japan, **Chair and Organizer of the Genes to Protein Session** and speaker
2008 RNA and Disease, Rome, Italy
2013 CurePSP 2013 International Research Symposium, Baltimore, MD

Invited National Seminars

1988 RNA Processing Meeting, Cold Spring Harbor Laboratory
1989 Albert Einstein Medical Center, New York
1989 St. John's University, New York
1989 Thomas Jefferson Medical Center, Philadelphia
1989 National Institutes of Health, Bethesda
1989 Bowman Grey Medical Center, North Carolina
1989 Lehigh University, Pennsylvania
1990 RNA Processing Meeting, Cold Spring Harbor Laboratory
1990 Midwest Yeast Meeting, University of Chicago
1991 Lake Forest College, Illinois
1993 Midwest Yeast Meeting, University of Chicago
1993 Cold Spring Harbor Laboratory in the Molecular Genetics, Cell Biology & Cell Cycle of Fission Yeast
1993 Biological Chemistry Department, Chicago Medical School

1994 Microbiology & Immunology Department, Chicago Medical School
1995 Department of Molecular & Cellular Biochemistry, Loyola University
1995 Pioneer Hi-Bred International, Inc., Iowa
1995 GeneMedicine, Texas
1996 Illinois Institute of Technology, Chicago
2000 Milwaukee College of Medicine, Wisconsin
2001 Northwestern University Medical School, Chicago
2002 Women in Science Issues Roundtable at the RNA Meeting, Madison WI
2002 State University of New York Health Science Center at Brooklyn
2004 Second Annual Interdepartmental Neuroscience Retreat
2006 Microbiology & Immunology Department, Chicago Medical School
2007 Fifth Annual Interdepartmental Neuroscience Retreat
2008 Movement Disorder Clinic, Rush University
2010 RNA Club, Northwestern University
2011 Molecular and Cellular Science Seminar at Rosalind Franklin University of Medicine and Science
2011 Interdepartmental Neuroscience and Neuropharmacology Retreat 2011
2012 Parkinson's Disease Models, Biomarkers, and Biochemical Pathways, New York City
2012 Neurodegenerative diseases, Cold Spring Harbor Laboratory
2013 Grand Challenges in Parkinson’s Disease, Grand Rapids, MI